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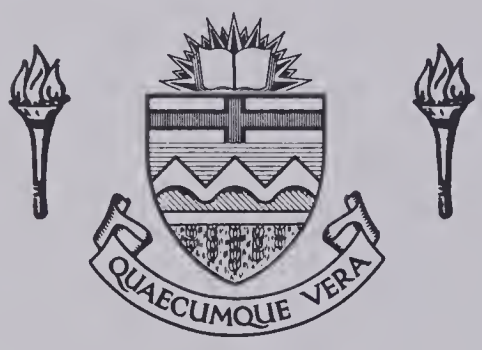

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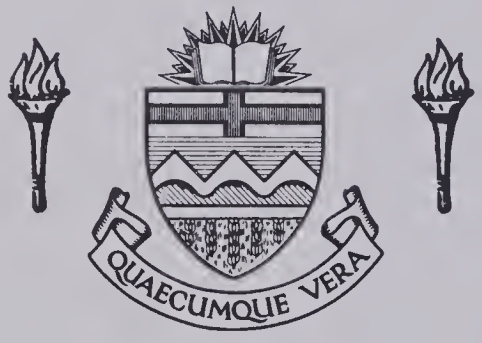

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A LIGHT AND ELECTRON MICROSCOPE STUDY OF THE CAT CARDIAC GANGLIA

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled A Light and Electron Microscope Study of the Cat Cardiac Ganglia submitted by Joan Gaunce in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Groups of cardiac ganglion cells are found in the fatty connective tissue beneath the pulmonary artery where it passes over the interatrial septum. Ganglia were excised from this area after the heart had been fixed by in situ perfusion with glutaraldehyde. The pieces of tissue were subsequently post-fixed in osmium tetroxide and embedded in Epon or paraffin previous to sectioning for light or electron microscopy.

The ganglia were found to contain ganglion cells, Schwann or satellite cells, cells containing dark granules, and connective tissue elements. An unusual cytoplasmic feature of some of the ganglion cells is a membrane complex resembling a glycogen array. The Schwann or satellite cell capsule surrounding the ganglion cell is in the form of loose myelination, being composed of layers of satellite cell cytoplasm wrapped around the ganglion cell. The cells containing dark granules are smaller than the cardiac ganglion cells. Long processes, also containing dark granules have been seen originating from these cells. Within the ganglion, nerve varicosities are found containing agranular vesicles, small granular vesicles and, in some varicosities, both types of vesicles.

Light microscopy studies using glutaraldehyde- and osmium tetroxide-fixed tissues show that cells containing norepinephrine are present in the cardiac ganglia. The characteristics of these cells seem to identify them as the cells containing dark granules seen under the electron microscope.

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INTRODUCTION

There has been much controversy about the distribution and role of autonomic nerve cells and fibers within the heart. Extrinsic and intrinsic cardiac ganglia have been described by light microscopists and it has been claimed that the cardiac ganglia are entirely parasympathetic (Woollard, 1926). On embryological grounds, it was suggested by Kuntz (1909) and by Mitchell (1956) that neurons of either afferent or efferent type could be present in the cardiac ganglia. There is undoubtedly a very close anatomical connection between sympathetic and parasympathetic cardiac nerves as they approach the heart (Nonidez, 1939) and recent investigations into the relationship of chromaffin cells and adrenergic nerve fibers to cardiac ganglia of several species (Jacobowitz, 1967) have shown the presence of fluorescing cells in the cat cardiac ganglia, some of which have long processes similar to neurons.

While there have been a number of electron microscope studies on sympathetic ganglia (Barton & Causey, 1938; Wyburn, 1958; Elfvin, 1963a, b; Grillo, 1966) those on parasympathetic ganglia are fewer in number. This is particularly true of those ganglia which are buried in the tissue or organs which they innervate, such as the cardiac ganglia. This study was designed to elucidate the ultrastructure of the cardiac ganglia, to describe the characteristics of the cells of which the ganglion is composed, and to investigate by morphological means, the relationships between some of these cells.

In the parasympathetic system, where the ganglia are often located in the tissues innervated, a release of catecholamines, after parasympathetic preganglionic stimulation, either into the synaptic space or into the general area of the ganglia, could have considerable altering or modifying effects on the transmitter function of the ganglion. In addition, since individual nerve fibers are difficult to identify with the light microscope, there may be some so far unobserved nerve fiber connections between these catecholamine-containing cells and both the preganglionic fibers and the postsynaptic ganglion cell. Therefore the observation of what appeared to be chromaffin-type cells among ganglion cells made while investigating the cardiac ganglia of a cat under the electron microscope, indicated that it may be of importance to use the electron microscope to look for anatomical and possibly functional connections between the cardiac ganglion cells and their catecholamine-containing cells.

HISTORICAL REVIEW

There is a large amount of literature on the discovery and development of concepts about the autonomic nervous system, the neuron, the terminal reticulum and the ganglia, of which only the latter is under consideration here. Only recently have chromaffin-type fluorescing cells entered the picture of the autonomic ganglia, particularly the parasympathetic branch. Also, the study of the satellite or Schwann cell has proceeded independently, as it is related not only to the autonomic system but to other nervous tissues as well. Thus, in the interests of clarity, it appeared wisest to review the history of research into the parasympathetic cardiac ganglia and some of its components separately. The possible interrelations and the significance of their forms as found in the cardiac ganglia will be discussed later in the discussion at the end of this thesis.

GENERAL

Parts of the autonomic nervous system have been described since the days of Galen in the 4th Century A.D. (Kuntz, 1953a) and it was in 1800, well over a century ago, that Bichat, described two separate nervous systems corresponding to the somatic and visceral systems of today. Many physiologists of Bichat's day thought that the visceral or vegetative system was isolated from higher nervous centres and the ganglia of this system were thought to be independent nerve centres. The true nature of ganglia was not recognized until Remak (1838) realized that nerve fibers arose from ganglia, thus giving the first picture of ganglia as they are seen today. Subsequently work by Bernard (1878), Langley (1898), and Gaskell (1916) developed the concept of the autonomic system with divisions into two subsystems that act in opposition to one another and which, through reflex actions, control visceral functions. Langley (1905) further contributed to our present knowledge when he discovered the effects of nicotine on ganglia and used it to map out the ramifications of much of the autonomic nervous system. He also gave the two systems their presently used names; the sympathetic and parasympathetic systems.

What is now considered to be the classical view of the autonomic nervous system was established early in this century with its two divisions clearly defined on the basis of anatomical distribution, physiological effects and postganglionic transmitter substances. Ganglionic transmission was considered to be purely cholinergic and the ganglia were viewed as simple synapses between preganglionic nerve terminals and the cell bodies of the postganglionic neurons. Although some controversy existed regard-

ing the nature of terminal innervation of organs by the autonomic nervous system, i.e. whether or not there is a terminal reticulum, the ganglia were, until recently, considered to be a collection of cell bodies of efferent neurons, innervated by preganglionic fibers arising from cell bodies in the central nervous system. The action occurring at the ganglion was considered to be that of a relay station where a relatively small number of preganglionic neurons stimulated a larger number of post-ganglionic neurons via the release of acetylcholine. In contrast to the somatic system where the cell bodies of efferent neurons are located in the spinal cord and subject to a complex array of neuronal connections the autonomic ganglia were believed to be conveniently isolated from complex factors and responding only to preganglionic impulses and humoral influences arriving through the blood stream. This classical picture now appears to be much too simple a view of autonomic transmission. Although acetylcholine has been well established as the main transmitter substance involved in autonomic ganglia (Dale, 1933) the development of the histochemical fluorescent technique (Eranko, 1964; Falck & Owman, 1965) for microscopic observation of catecholamines in tissues, has revealed the presence of fluorescing chromaffin-type cells in and near autonomic ganglia in which transmission had formerly been considered to be purely cholinergic (Norberg & Hamberger, 1964; Eranko and Harkonen, 1965; Jacobowitz, 1967, 1970; Jacobowitz and Woodward, 1968). Chromaffin tissue is known to be widely distributed as the paraganglia (Leeson & Leeson, 1966) which are often associated with the sympathetic ganglia. Coupland (1965a) comments briefly on the possible function of extra adrenal chromaffin tissue without coming to any firm conclusions about

its role. The presence of chromaffin-type cells in autonomic ganglia has been also confirmed with electron microscope studies (Grillo, 1966; Elfvin, 1968; Siegrist et al., 1968; Jacobowitz, 1970).

Hoffman et al., (1945) found in atropinized dog that large doses of acetylcholine cause a stimulation of heart rate which was abolished by nicotine. They assumed that nicotine acted on either sympathetic ganglia or chromaffin tissue in the heart. Kottegoda (1953) also suggested that the cardiac ganglia are capable of liberating considerable quantities of an adrenaline-like substance. Burn (1960) following a series of investigations postulated a new adrenergic mechanism which involved the release of noradrenaline from peripheral stores by the action of acetylcholine. Norberg and Hamberger (1964) in their study of the peripheral adrenergic neurone found no support for a storage site other than the adrenergic nerve. They found noradrenaline stored throughout the nerve cell, not just in the nerve terminal, though the concentration of transmitter was found to be much higher in the terminals. The adrenergic terminals were found throughout the autonomic ganglia. An interesting finding in that study was the presence of a few cells in sympathetic ganglia, located in clusters and much smaller than ganglion cells and exhibiting a different fluorescence than the ganglion cell. They make no mention of chromaffin cells.

THE AUTONOMIC NERVOUS SYSTEM

Kuntz (1953b) mentions the earliest description of ganglion cells as having been made by Ehrenberg in 1833 in the early days of use of the light microscope. It was recognized before that time that the

ganglia played a role in the autonomic system, but no one postulated that fibres arose from the ganglia. Remak in 1854 made an extensive study of the sympathetic ganglia which contributed much to the understanding of the ganglionic structure and discovered the ganglia of the turtle heart which now bear his name.

The concept of the nervous reflex with central nervous centres was established with Claud Bernard's (1878) studies. Gaskell in 1886 further investigated the role of different portions of the autonomic nervous system, mentioning the difference between the sympathetic ganglia located some distance from the structures they innervate and the parasympathetic ganglia, which are located in or near the structures they innervate. The difference between sympathetic and parasympathetic systems was further emphasized by Langley in 1903, who stressed the latter's local effects as compared to the wide reaching effects of the former. Following the work of Gaskell and Langley it was accepted that nerve fibres ran into the ganglia in the autonomic nervous system and there made contact with nerve cells of the ganglia whose axons then innervated the peripheral tissue. Langley (1903) assumed that there were no 'connecting cells' in the ganglia, that influenced the efferent cells, other than the incoming preganglionic fibres. This view is now to be questioned with the discovery of neuro-humoral-type cells in the autonomic ganglia which appear to have functional connections with the ganglion cells. Possibly it is also true that the ganglion cells or incoming preganglionic fibres have functional connections with these neuro-humoral cells.

THE CARDIAC GANGLIA

The general structure and mode of functioning of the autonomic nervous system having been established by about 1920, more attention was then paid to the innervation of specific structures. Woollard (1926) investigated the innervation of the heart using the vital methylene blue technique, and found, as others before him, many groups of cardiac ganglia of varying sizes scattered over the atria. He found very few in the ventricles. The ganglia were mainly located sub-pericardially, and around the roots of the great vessels, and on the posterior surface of the atria. It was thought that there was a capsule around individual ganglion cells and pericapsular endings were described where the preganglionic nerve terminal appeared to be applied to the capsule. Pericellular endings appeared to be applied to the nerve cell body or its dendrites. Different types of nerve cells were described in the ganglia, and Woollard described four types according to structural characteristics, one of which was multipolar with a non-myelinated axon, and another of which was unipolar. De Castro (1932) had also separated them according to size - ranging from 15 to 60 μ in diameter. Embryological evidence prompted Kuntz (1909) to suggest that cardiac ganglia belonged to both sympathetic and parasympathetic systems and others have felt that both sympathetic and parasympathetic neurons terminate in the cardiac ganglia. The detailed studies by Dogiel (1877), Cajal (1891) and de Castro (1932) on the structural characteristics of the ganglion cells, their arborizations and neuronal terminations, apart from indicating some unknown difference in function, do not serve to elucidate much about the events occurring in a ganglion,

as we now know it. One might speculate that the rarely found bi- or unipolar cells in the ganglia described by these authors could be the fluorescing or neuro-humoral cells as these latter cells occur in much less abundance than the ganglion cells themselves. Or perhaps more likely the Type V cells as quoted by de Castro (1932) and described as possessing few dendrites, measuring 15 to 34 μ in diameter and often containing large amounts of argentophile pigment, might in fact be these neuro-humoral cells.

THE CHROMAFFIN CELL

The chromaffin cell has been defined by Coupland (1965b) as being an element developed from neuroectoderm, innervated by preganglionic sympathetic nerve fibres and capable of synthesizing and secreting catecholamines. A chromaffin cell by definition should also store catecholamines in sufficient quantity to give a positive chromaffin reaction with aqueous solutions of potassium dichromate. They were first recognized by Stilling in 1870 as brown coloured cells of the dichromate-fixed adrenal medulla, and of the tissue associated with the abdominal paravertebral sympathetic plexes of cat, dog and rabbit. Oliver and Schafer wrote of the physiological action of adrenal extract as early as 1894.

The classical fixative producing the chromaffin reaction was formol dichromate. The reaction involves the oxidation of adrenaline and noradrenaline at neutral pH and the subsequent production of adrenochrome and noradrenochrome.

Gaskell in 1920 mentions the connection between chromaffin cells (as identified by their chrome - staining reaction) and the sympathetic

nervous system. It is known that in the embryo the early chromaffin cells cannot be differentiated morphologically from other primitive forms of sympathetic system cells (Coupland, 1965c). Gaskell (1920) also notes that in the lowest vertebrates there are few sympathetic cells and many chromaffin cells, while in amphibia, chromaffin cells and sympathetic cells are mixed in ganglia. In the mammal he thought that chromaffin cells were not usually found in sympathetic ganglia. However, chromaffin cells have been found in many tissues. Boyd (1960) mentions the presence of chromaffin cells in the adrenal medulla, abdominal paraganglia, organ of Zuckerkandle, vagal paraganglia and a number of other sites. Within the past fifteen years interest in chromaffin cells has heightened. Coupland (1960d) found chromaffin cells to be widely scattered in adult rabbit, mouse, guinea pig, cat and man in the same regions they are found neonatally.

Since the advent of the technique of distinguishing catecholamines using the fluorescent microscope reported by Eranko (1964), Falck and Owman (1965), histochemical studies using this technique (Eranko & Harkonen, 1965; Hamberger, Norberg & Sjoquist, 1965; Norberg & Sjoquist, 1966) sometimes combined with acetylcholine esterase staining (Jacobowitz, 1967) have clearly shown the presence of cells and their processes containing catecholamines in additional sites. Much interest has centered on those found in ganglia. It appears that workers are now less concerned with the chromaffin reaction than previously and choose to identify these cells by their fluorescent reaction or by the osmophilic reaction of their granules, although they still call them chromaffin cells (Siegrist

et al., 1968); and acknowledge that they are in fact intermediate in characteristics between the chromaffin cells of the adrenal medulla and those of the sympathetic neurons. In this thesis the cells which have similar characteristics to the fluorescent, or dark granule-containing cells mentioned above are termed chromaffin-type cells.

THE SCHWANN OR SATELLITE CELL

The cell surrounding the nerve axon was first described by Theodore Schwann in 1839 (Causey, 1960) and was later named after him. Causey claims the term Schwann cell to be used for any cell that surrounds a nerve fibre with its cytoplasm. Gasser in 1952 was the first to recognize the mesaxons in Schwann cells surrounding unmyelinated nerve fibres, and later Geren (1953) described the mode of formation of the myelin sheath in developmental studies.

Nageotte (1932) considered that Schwann cells formed a syncitium; Barton and Causey (1958) also considered the possibility of the continuity of the cytoplasm around contiguous satellite cells and around nerve cells in the superior cervical ganglion.

Luse in 1958 reported that the satellite cells of ganglia often show infoldings of the cell membrane which resemble myelinization. She thought that the Schwann cells of the peripheral nerve are continuous with those surrounding the ganglion cells, and that the satellite cells form myelin around the neurons of the vestibular ganglion.

GLYCOGEN MEMBRANE COMPLEXES

A brief survey of the literature on the glycogen array must be included in the review of literature pertinent to this study, as a

structure more closely resembling the descriptions of this membrane complex than any other, has been seen during this investigation of the cardiac ganglion.

The occurrence of glycogen membrane complexes has been reported in as widely differing cells as rat liver (Flak, 1968), fungi (Calonge, 1969) and mouse striated muscle (Garant, 1968). The latter example is the one offering the greatest similarity to the membrane granule complex found in the cardiac ganglion cells. Nonetheless the general description of granules of glycogen arranged in an orderly fashion between layers of membranes situated intracellularly applies to all cases.

In 1968 Garant reported a membrane complex in mouse striated muscle cells, situated peripherally in the cell, and frequently closely associated with mitochrondria and glycogen stores. The structure was seen as a circular, semicircular or flat arrangement of what he terms 'smooth membraned cisternae' and narrow cytoplasmic bands. Each cisterna is described as a pair of parallel membranes 300 to 450 Å apart, with fine granular material in the intervening space. Between the cisternae he found glycogen particles 250 to 350 Å in diameter in single file. Sometimes rosettes of glycogen were seen near the periphery of the array.

METHODS

The hearts of seven anaesthetized cats were perfused in situ retrograde via the abdominal aorta with a solution of 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C for 10 to 20 minutes. The whole heart with stumps of accompanying vessels was then cut out of each animal, and further fixed in the same glutaraldehyde solution overnight or longer. This was found to produce a good fixation of the tissue as seen by the condition of the mitochondria. Subsequently small pieces of fatty connective tissue and muscle, reported to be rich in ganglion cells by Calaresu and St. Louis (1968) were cut out from the area beneath the pulmonary artery where it passes over the interatrial septum. This tissue was then rinsed in 10% sucrose in phosphate buffer (ph 7.3) at 4°C for one or two hours. If the tissue needed to be stored for any length of time it was then stored in fresh 10% sucrose in phosphate buffer in the refrigerator in a covered jar. For subsequent processing tissues were cut into small pieces not more than 1 mm thick, post-fixed in phosphate buffer containing 1 - 2% osmium tetroxide, dehydrated in alcohol, and embedded in Epon according to the method of Luft (1961). Thick survey sections (about 1 μ) were cut with glass knives on a Porter-Blum ultramincrotome, until groups of ganglion cells were located by viewing the sections under phase contrast microscopy, or by staining with Richardson's stain (Richardson, et al. 1960) as follows, and viewing under bright field microscopy. Sections 1 μ thick were mounted on a slide by placing them in a drop of water on the slide and heating the slide at 60 - 80°C to flatten and dry the sections. A drop of 1% periodic

acid was added and left on for 5 minutes at room temperature. The slide was washed well in distilled water, and blotted dry with lens tissue. Then a pool of Mallory's Azur II - methylene blue, made by taking equal volumes of 1% Azur II in distilled water and 1% methylene blue in 1% borax solution, was dropped on the sections. The slide was warmed for 5 minutes on a hot plate, then the excess stain was rinsed off with distilled water. The preparation was dried by reheating, then immersed in xylene, drained and mounted in Permount.

From the main block thin sections (silver interference color) were then cut, double stained with 40% uranyl acetate in methanol, and 0.7% aqueous lead citrate, and viewed in a Philips EM 100 at accelerating voltage 60 KV, or in a GEM 50 at accelerating voltage at 60 KV. Electron-micrographs were taken onto 35 mm film in the Philips, and onto 4 x 5 plates in the GEM.

To reduce the time required for cell survey of blocks of Epon-embedded tissue by 1 μ sections in search of groups of ganglion cells, another method was developed for a period of time with some success. This involved cutting 5 - 20 μ sections of Epon-embedded tissue on a Leitz rotary microtome as one would cut paraffin sections. It was found possible to cut serial sections of the tissue using an ordinary microtome knife. The edge of the knife became somewhat damaged in the process, but as the size of the block was very small, one knife lasted sufficiently long to make the technique practical, and the knife edge could be reground when necessary. As each section was cut it was mounted with Permount on a microscope slide, and viewed by phase contrast micro-

scopy. Thus the locating of clumps of ganglion cells was speeded up considerably. An additional advantage of this method is that due to the glutaraldehyde - OsO_4 fixation and staining, the noradrenaline-containing granules in chromaffin-type cells appeared dark and these cells could be identified at the light microscopy level previous to electron microscopy viewing. Sections of 5 - 8 mm square could be cut on the rotary microtome for surveying. When the desired ganglion cells had been located the block could then be trimmed to a size suitable for ultrasectioning, the location of the cell on the face of the block being readily found by comparing the block face and the slide showing the ganglion cells under a dissecting microscope.

Subsequently, another method of locating groups of ganglion cells was used, as described by Cookson (personal communication). Six formalin-fixed rat hearts were used for a trial of this method, which was later applied to portions of glutaraldehyde-fixed cat heart. The fixed tissue was frozen and sectioned in a Lipshaw cryostat, stained with 1% methylene blue, and examined under a light microscope for the presence of clumps of ganglion cells. These were easily visible at 400 - 500 X magnification. From the frozen heart it was then easy to locate the site of the ganglion cells by viewing the cut face of the tissue under a dissecting microscope beside the slide of the sections in which the ganglia could be seen. Small pieces of tissue containing the ganglion cells could then be excised with a small pair of sharply pointed scissors, and placed in 10% sucrose in

phosphate buffer for rinsing prior to post-fixation in OsO_4. The subsequent treatment was identical to that previously mentioned.

OBSERVATIONS

LIGHT MICROSCOPY

As reported by Woollard (1926), groups of ganglion cells were found around the roots of the great vessels, and in the area of the interatrial septum. The size of these groups varied from a very few cells (Fig. 1) to a large number of ganglion cells (Fig. 2) in one group. The ganglion cells at the light microscopy level of magnification showed encapsulation (Fig. 1, 3, 4 & 5) as mentioned by previous authors (Woollard, 1926; Valentin, 1936) as well as connective tissue interspersed between them in varying amounts. The encapsulation was visible as a material surrounding each ganglion cell, which was associated with an elongated nucleus lying near the ganglion cell body (Fig. 5). Frequently, more than one of these nuclei could be seen in the capsule. Further details of the encapsulation are visible only under the greater magnification of the electron microscope, as will be described later, where the 'capsule' is much different in appearance compared to the light microscope.

The ganglion cells were found to stain darkly (with thionine) (Fig. 1, 2 & 3) in the cytoplasm, while the nuclei remained paler. Mitochondria are visible at high magnification (Fig. 5). In contrast the satellite cell nuclei stained dark blue with thionine, while the cytoplasm remained light enough to prevent a good view of the cell.

In the 1 μ Epon sections stained with Richardson's stain (Richardson et al., 1960), the ganglion cells stained a medium blue with a lighter blue nucleus (Fig. 4 & 5). Again the satellite cell

nucleus stained a dark blue, but the cytoplasm took up very little stain, making the edges of these cells very difficult to define using light microscopy.

While surveying 5 μ Epon sections cut on a rotary microtome, cells having a dark appearance were observed (Fig. 6 & 7). The only material with staining properties to which the tissue had been exposed was osmium tetroxide. These dark cells were 5 - 10 μ in diameter - appearing obviously smaller than the ganglion cells, but found among them, either singly (Fig. 8) or in small clusters (Fig. 9). They appeared to have long processes which were also dark in appearance (Fig. 6 & 8). On closer observation the dark material was resolved as darkly stained granules found in the cytoplasm (Fig. 8). The nuclei of these cells appeared pale, as did the ganglion cells, so that the dark granule-containing cells stood out quite markedly.

The cardiac ganglia were frequently seen to be highly vascularized (Fig. 4, 6 & 7). Many capillaries were seen to thread throughout the ganglion, and some of the dark granule-containing cells were positioned immediately next to a capillary, while others were positioned within the body of the ganglion not apparently contacting a capillary (Fig. 10 & 11).

LIGHT MICROSCOPY CONFIRMATION OF CATECHOLAMINE CONTAINING CELLS

As the chromaffin-type cells containing darkly staining granules were first seen in this study in the electron microscope, it was thought necessary to confirm their presence, using the method of Coupland, Pyper and Hopwood (1964) in wax sections. Thus, glutaraldehyde

fixed, and OsO_4 post-fixed tissue, from the same hearts used above, was dehydrated through the alcohols, embedded in paraffin, sectioned at 8 μ and mounted on glass slides with gelatin. After drying the slides were deparaffinized in xylene, and mounted in permount (Fig. 12, 13 & 14). Some light brown and some dark brown cells were seen under the microscope.

In addition to the above techniques, sections of formalin fixed, paraffin embedded tissue of pieces of cat heart and whole rat hearts were stained by the thionine method (Fig. 1, 2 & 3) (Clark & Sperry, 1945), and viewed by light microscopy to confirm the position of the ganglion rich areas.

All of the tissues used were confined to the atria of the heart, on the surface from which the great vessels arise, reportedly an area rich in cardiac ganglia (Woollard, 1926).

ELECTRON MICROSCOPY

Under the greater resolution of the electron microscope, the cardiac ganglia appear to consist of four types of cells, namely the ganglion cells (presumably parasympathetic) (Fig. 14), satellite or Schwann cells (Fig. 14), chromaffin-type cells containing dark granules (Fig. 15), and fibroblasts (Fig. 15). Collagen is also present in the intercellular spaces (Fig. 15). The endothelial cells of the capillary walls are also viewed within the tissue on some slides (Fig. 16).

THE GANGLION CELL

The ganglion cells range from 15 to 40 μ in diameter, and

each one contains a large central nucleus and nucleolus (Fig. 15). The cytoplasm contains numerous mitochondria with regularly arranged transverse cristae. The mitochondria seem to be dispersed evenly throughout the cytoplasm, and into the cell projections. There is present endoplasmic reticulum, Golgi bodies, and neurotubules (Fig. 17), the latter of which are particularly obvious where a dendrite projects from the cell body (Fig. 18). Numerous dense bodies can be seen, as well as occasional multivesicular bodies (Fig. 17). One unusual feature noticed in the cytoplasm of some ganglion cells is the presence of a very highly organized structure of dense granules arranged in an orderly fashion between membranes about 250 - 400 Å apart (Fig. 19 & 20). The granules measure 150 - 300 Å in diameter and are not always attached to the membranes. The granules are sandwiched between rows of membrane. In various places the granules may touch one or another or both of the membranes, or neither, but appear to be positioned in between the membranes making no contact (Fig. 19, 20 & 21).

THE SCHWANN OR SATELLITE CELL

Typical Schwann cells are seen in the ganglion in relationship to the efferent fibres of the ganglion. The unmyelinated axons can be seen embedded in Schwann cell cytoplasm, the mesaxons indicating the site of invagination of the cell membrane (Fig. 22 & 23). Myelinated fibres are seen enveloped in their many layered sheath and passing close to the Schwann cell nucleus (Fig. 24). Under low power electron microscopy, Schwann or satellite cell nuclei can be seen in close relationship

to the ganglion cells, and it is apparent that an extension of the Schwann or satellite cell extends around the body of the ganglion cell. It proved to be rather difficult to obtain a clear picture of the nature of the enveloping sheath as in most low power pictures it appears as an indistinct grey edging. On looking under higher magnification at thin sections, the material surrounding the ganglion cell and its processes resolves into thin cytoplasmic layers which envelop the cell and its processes (Fig. 26). The basement membrane extends around the outside of this layer. The cytoplasmic sheets surround the various cell processes within the ganglion (Fig. 27) and also the synaptic terminals, though they are absent at the site of a synapse (Fig. 27).

CHROMAFFIN-TYPE CELLS

Chromaffin-type cells in small groups or singly appear among the cardiac ganglion cells. They are smaller cells which, when the tissues are fixed in glutaraldehyde, and post-fixed in osmium tetroxide, contain many darkly staining granules (Fig. 28 & 29). These cells are oblong in shape and have been seen with long processes (also containing dark-staining granules) extending from the cell body (Fig. 30). The cells have a large nucleus, some mitochondria, which have transverse cristae, and rough surfaced endoplasmic reticulum. They also appear to be surrounded by Schwann or satellite cell membranes. The darkly staining granules within the cells vary in size from about 1,000 Å to 2,500 Å in diameter. They appear to have a very dense large core

surrounded by a small less dense area just inside the membrane surrounding them. Within the population of dense-cored granules there are also a small percentage which are smaller, less dense, appearing grayish in the electronmicrographs, and as if containing much smaller granules (Fig. 31).

CELL PROCESSES FOUND IN THE GANGLION

Within the confines of a group of cardiac ganglion cells can be found cell processes, some probably arising from cells within the ganglia and others undoubtedly arising from outside the ganglion. These observations were principally concerned with the elements making synaptic contacts in the ganglion. Typical parasympathetic synaptic endings can be found in the ganglion. They contain agranular vesicles, about 300 Å in diameter and mitochondria (Fig. 27 & 32). These endings or terminal branch swellings can sometimes be seen in synaptic contact with other tissue. At the place of synaptic contact a slight increase in density of the presynaptic membrane can be seen and a greater increase in density at the postsynaptic membrane as compared to the nonsynapsing membrane (Fig. 27). Included in this population of agranular vesicles are usually a few larger opaque granules with a denser core, and a small, less dense area just inside the membrane surrounding the vesicle. These larger, denser vesicles measure about 800 Å in diameter (Fig. 32).

As well as the expected agranular vesicle containing terminations, two other types of granule-containing processes are seen. One closely resembles the agranular-containing swellings in that they contain

mitochondria and agranular vesicles about 200 to 400 Å in diameter, but differing in that there were a clump of closely grouped granular vesicles also in the swelling. These granular vesicles seem to vary in density from appearing black to medium grey, and are smaller in diameter than the agranular vesicles. They measured about 150 to 300 Å in diameter (Fig. 33). The third type of process contains only the granular vesicles, which appeared the same as the other granular vesicles. That is to say, they are 200 Å in diameter and varying from very dense or black in appearance to moderately dense or grey (Fig. 34 & 35). Similar granular vesicles were also seen in the cytoplasm of a cell protrusion (Fig. 35).

INTERRELATIONSHIPS BETWEEN CELLS AND CELL PROCESSES FOUND IN THE CARDIAC GANGLION

The ganglion cell, the chromaffin cell and the Schwann or satellite cell:

The Schwann or satellite cell is found in close proximity to the ganglion cell body, its processes and other processes found throughout the ganglion. Long thin extensions of satellite cell cytoplasm are found wrapped in layers numbering from three to eight, around the body of the ganglion cell and only on the outside of these is found the basement membrane. As processes leave the ganglion cell, they too are wrapped in Schwann cell extensions which continue around the sites of synaptic contact except at the points of synapse. The chromaffin-type cell appears also to be enveloped in the same way. The layers of satellite cell cytoplasm are 200 to 800 Å thick and about 150 to 250 Å

apart, in contrast to true myelination found in the postganglionic axons where the membranes are much closer (Fig. 22, 23, 24, 25, 26 & 27).

SITES OF VESICLE-CONTAINING SWELLINGS AND SYNAPTIC CONTACT

A. Agranular Vesicle Terminations:

The agranular vesicle terminations are found in synaptic contact with processes extending from the cardiac ganglion cells. These synapses occur mainly near the cell body (i.e. 1 to 10 μ away) (Fig. 27 & 34). No definitively identified instances of contact with the cell body were seen, though nerve terminals containing agranular vesicles were seen near cell protrusions. An instance of synaptic contact with an unmyelinated axon was recorded (Fig. 36). The agranular vesicle terminations were most commonly seen clustered around the area where a process left a ganglion cell body (Fig. 33 & 34). They were also seen apparently in synaptic contact with the body of chromaffin-type dark granule-containing cells (Fig. 29 & 30). Swellings containing agranular vesicles and mitochondria are seen in the tissue spaces between other tissues and not in synaptic contact (Fig. 32 & 35).

B. Other Vesicle Terminations:

Both other kinds of vesicle containing swellings were seen in close proximity to the ganglion cell body, frequently where a process was extending from the cell body. It appeared that the other terminations were approaching synaptic or at least close contact with the ganglion cell processes and/or the preganglionic endings which were in close relationship with them (Fig. 18, 29, 34 & 35).

DISCUSSION

The cardiac ganglion has been seen to contain three major cell types apart from the connective tissue elements. That is to say, the parasympathetic postganglionic cell, the Schwann or satellite cell and the dark granule-containing chromaffin-type cell. As well as these cell bodies, there are the axons and the dendrites of the ganglion cells, long processes from the chromaffin-type cells and axons and terminal branches from the preganglionic cells situated higher up in the nervous system.

The cardiac ganglion cells themselves seem to have one unusual feature - that of a whorl-like configuration of membranes and granules. This membrane complex closely resembles the glycogen array reported by Garant (1968) in striated muscle in size of granules, distance between membrane layers and in general arrangement of these components. They have been seen in the cardiac ganglion cells in circular, semi-circular or flat arrangement, as Garant has described in muscle. The complex is most frequently seen close to the periphery of the cell and sometimes associated with the protrusion of a dendrite from the cell. In one case, a dendrite was seen in cross section with the glycogen membrane complex arranged in circular fashion concentric to its circumference. Mitochondria have been seen in close relationship to the complex. It would be valuable to carry out histochemical studies to confirm the nature of the substance of the granules.

Another feature of interest in the cardiac ganglion is the means by which the Schwann or satellite cell surrounds the ganglion cell, and also apparently the chromaffin-type cell. In some autonomic ganglia, the satellite cell simply abuts onto the edge of the ganglion cell and extends a long thick process around it, or the chromaffin-type cell (Elfvin, 1968; Siegrist et al., 1968; and Elfvin, 1963). However, the capsule surrounding the cardiac ganglion cells and chromaffin-type cells within the cardiac ganglion is composed of many layers of sheath cell processes. This many layered capsule is comparable to the loose myelination described by Rosenblueth and Palay (1961) in the eighth nerve ganglion of the goldfish. This loose myelination provides a better means of isolating the cell body from the extracellular space and possibly from tissue contacts in the ganglion. No synapses were definitely identified as occurring on the cell body of the parasympathetic neurons, though this was not true in the case of the chromaffin-type cells. In the latter, large areas of postsynaptic surface were found similar to those mentioned by Williams and Palay (1969). The ganglion cell dendrites and possibly the axons near the cell body, seem to be the site of greatest postsynaptic activity for these cells as frequently nerve swellings containing synaptic vesicles were seen clustered around a process as it arose from the cell body and wandered into the ganglion.

Chromaffin-type cells have been reported in fluorescent microscopy studies and light and electron microscopy studies in a

variety of ganglia, in a number of different animals. Siegrist et al. (1968) has seen them in the superior cervical ganglion of rats, Elfvin (1968) in the inferior mesenteric ganglion of rabbit, Norberg and Sjoquist (1966) in the stellate ganglion of rat, and Jacobowitz (1967, 1970) in a number of sympathetic ganglia and in the cardiac ganglia of cat. Only in the cat cardiac ganglia did Jacobowitz (1967) find cells which gave a positive chromaffin reaction, though in the other animals he found fluorescent cells which greatly resemble the chromaffin cells of the adrenal medulla. He found that most of the chromaffin cells are innervated by acetylcholinesterase staining nerve fibres. My findings support this as presynaptic endings were seen on the chromaffin-type cells which contained large numbers of agranular vesicles typical of preganglionic cholinergic terminals (Grillo, 1966). These cells are believed to have the function of interneurons within the ganglion, in some way modifying the response of the postganglionic cell to preganglionic stimulation. Most of the work on these interneurons has been done on sympathetic ganglia which are more readily accessible to study and experimentation. However, Burn and Rand (1965), believe there is present in heart tissue a storage depot of noradrenaline innervated by cholinergic fibres. It was also found that in the nictitating membrane of cat (Burn et al. 1959) the chromaffin cells became pyknotic with sparse granules and were reduced in number after denervation or reserpine treatment indicating that transneuronal degeneration had occurred.

Jacobowitz (1967) postulates that the cholinergic innervation of the chromaffin-type cells could be either pre- or postganglionic in origin. Indeed, it could be both. However, functionally, similar results would be obtained in that a negative feedback response could be initiated by stimulation of ganglionic transmission causing liberation of catecholamines in close contact with ganglion cell dendrites and/or preganglionic nerve branches, which by hyperpolarization would depress transmission. De Groat and Volle (1966) show that noradrenaline can depress or facilitate transmission in the cat superior cervical ganglion by bringing about a hyperpolarization or a depolarization of the ganglion cell membrane. Topchieva (1966) found hyperpolarizing responses in the cardiac ganglia of frog which were elicited by sympathetic stimulation. Also, she found there were occasional cells (about 10%) whose membrane potentials were increased, not reduced like the majority of cells by vagal stimulation. The degree of membrane hyperpolarization in these cases increased with the strength of vagal stimulation. Thus it is apparent that more responses than simple transmission of preganglionic impulses are possible within the cardiac ganglia and that hyperpolarization does occur probably causing a depression of transmission.

Because the chromaffin-type cells frequently seem to be localized around capillaries, it may be that catecholamines are released into the extracellular space, which then travel to other tissues either via the blood stream or by the extracellular space. Another possibility is that the dark granule-containing cells send out long processes in the

cardiac muscle itself. Thaemert (1966) shows very nicely the presence of granular vesicle- and agranular vesicle-containing terminations side by side making intimate contact with cardiac muscle cells in the frog ventricle. Jacobowitz (1967) shows fluorescent fibres in the region of the A-V node. Another possible function of these cells is to act on the cardiac blood vessels themselves. Considering their usual close position to capillaries, it is a role that must be considered.

It is interesting that in the synaptic contacts found on the chromaffin-type cell bodies, the area of soma that is in postsynaptic contact appears greater than other postsynaptic areas in the ganglion. This has also been suggested by Williams and Palay (1969). It may mean that the effect of preganglionic stimulation is enhanced. Rather than having to rely on summation of the effects of preganglionic stimuli in a larger number of terminals to achieve depolarization of the cell body, fewer preganglionic stimuli could cause cell membrane depolarization, and activate the cells. It can be argued that the chromaffin cells are small in number and therefore probably do not exert a profound effect on the ganglion. However, until the mode or modes of action of these cells is elucidated, no meaningful statement as to the significance of their small number or their function can be made. If, indeed, the chromaffin-type cells act by direct synaptic action on postganglionic nerve cells, by release of noradrenaline into the extracellular space of the ganglion and into the blood stream via the capillaries so close to which they are often found; and in this latter case that this release affects other tissues in the heart as well as the blood vessels themselves, then by means of many sites of activity it is certainly feasible

that these catecholamine-containing cells, despite their small number, could have a very important role in cardiac function.

SUMMARY

A histological and electron microscopic study of the cardiac ganglia has shown the presence of several unusual features. Membrane complexes resembling glycogen arrays are seen in the parasympathetic ganglion cells, particularly near the site where an axon leaves the cell body. The Schwann or satellite cell capsule around the ganglion cell is formed by the wrapping around the cell of layers of satellite cell cytoplasm 200 Å to 800 Å thick. Chromaffin-type catecholamine-containing cells are seen in the ganglia often associated with capillaries. They have long processes, and the soma and processes contain large osmophilic granules. As well as the usual agranular vesicle-containing nerve endings found in the ganglia, other endings are seen which contain dark granular vesicles, or which contain both agranular and granular vesicles. The possible significance of the presence of these catecholamine-containing cells in the cardiac ganglia is discussed.

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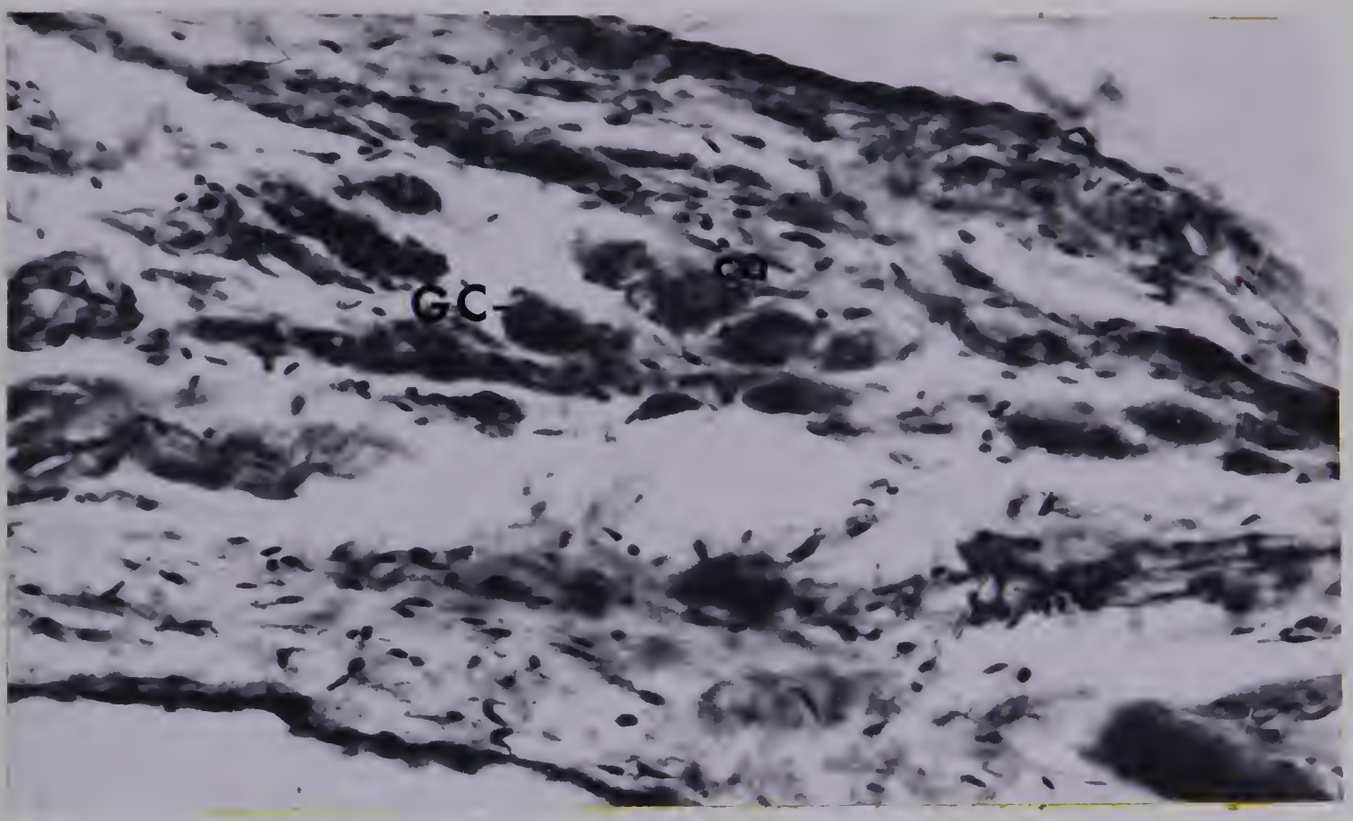
FIGURES

Figure 1. A low power photomicrograph of a small group of ganglion cells (GC) in cat, stained with thionine. Encapsulation is shown. The nuclei of the capsule cells (ca) surround the ganglion cell.

Thionine stain. Magnification x 320.

Figure 2. A photomicrograph of a large group of ganglion cells (GC) in rat heart. The nuclei of the capsule cells (ca) can be seen.

Thionine stain. Magnification x 320.



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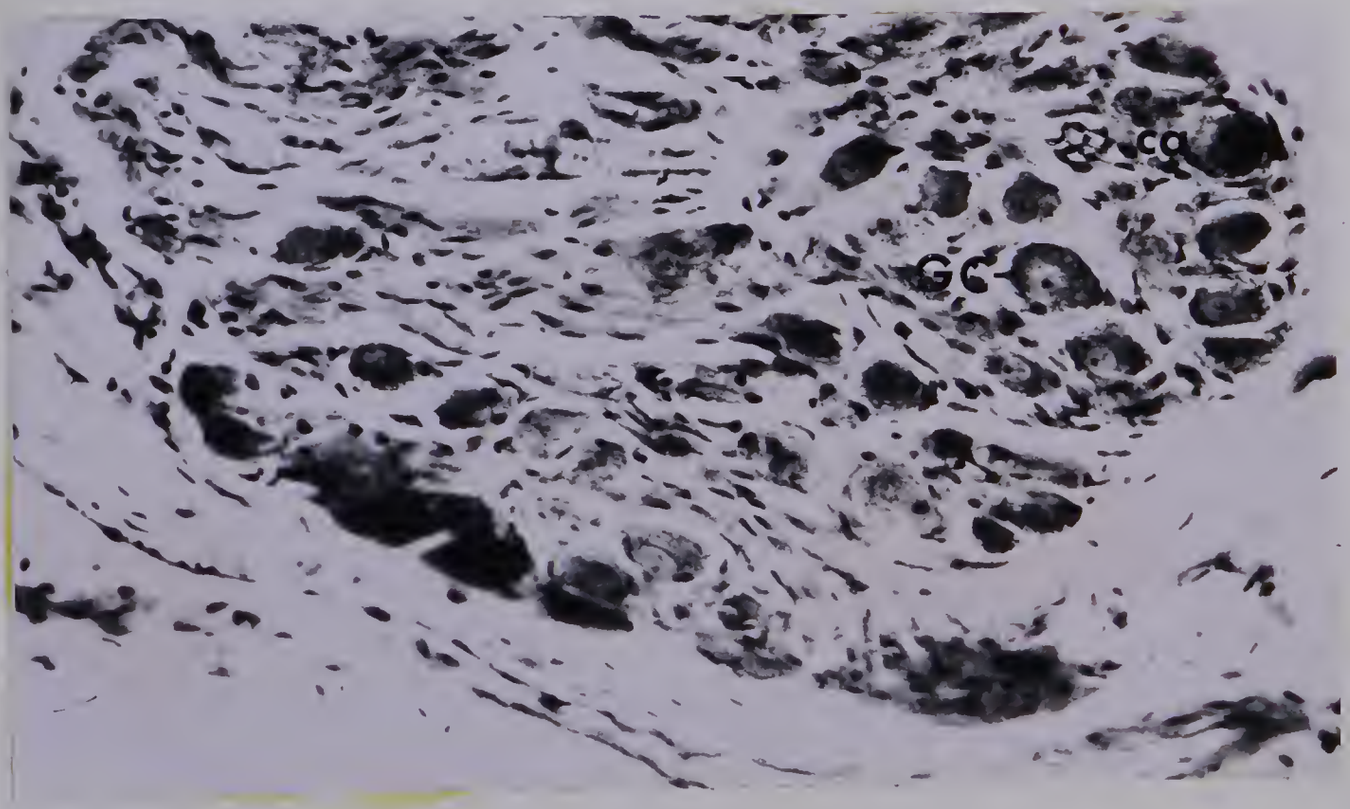

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Figure 3. A photomicrograph of ganglion cells stained with thionine. Paler staining nucleus (N) and darker staining cytoplasm (Cy) shown. Also visible are the nuclei of the satellite cells which form the capsule (ca) around the ganglion cell.

Thionine stain. Magnification x 2,000.

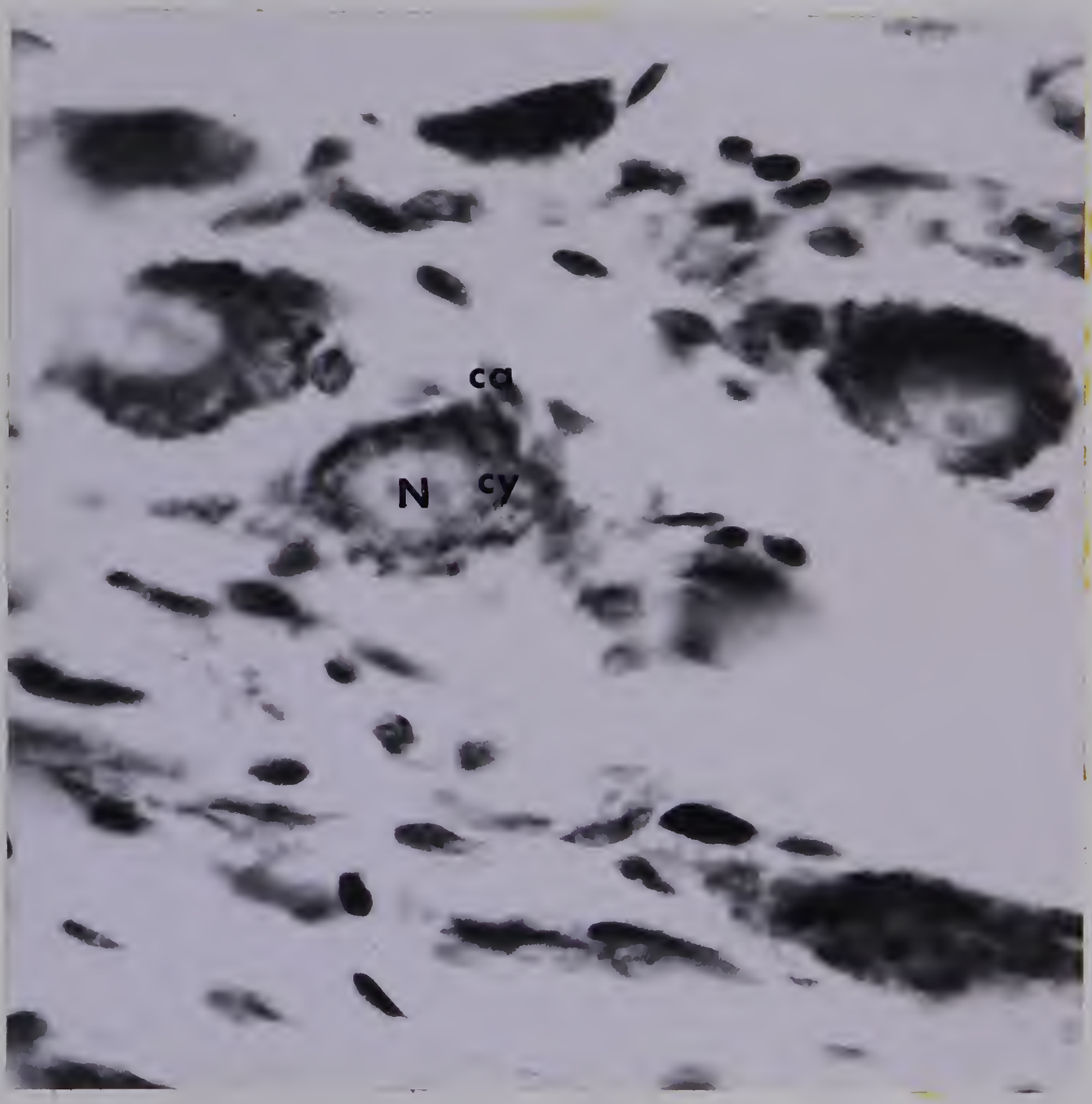

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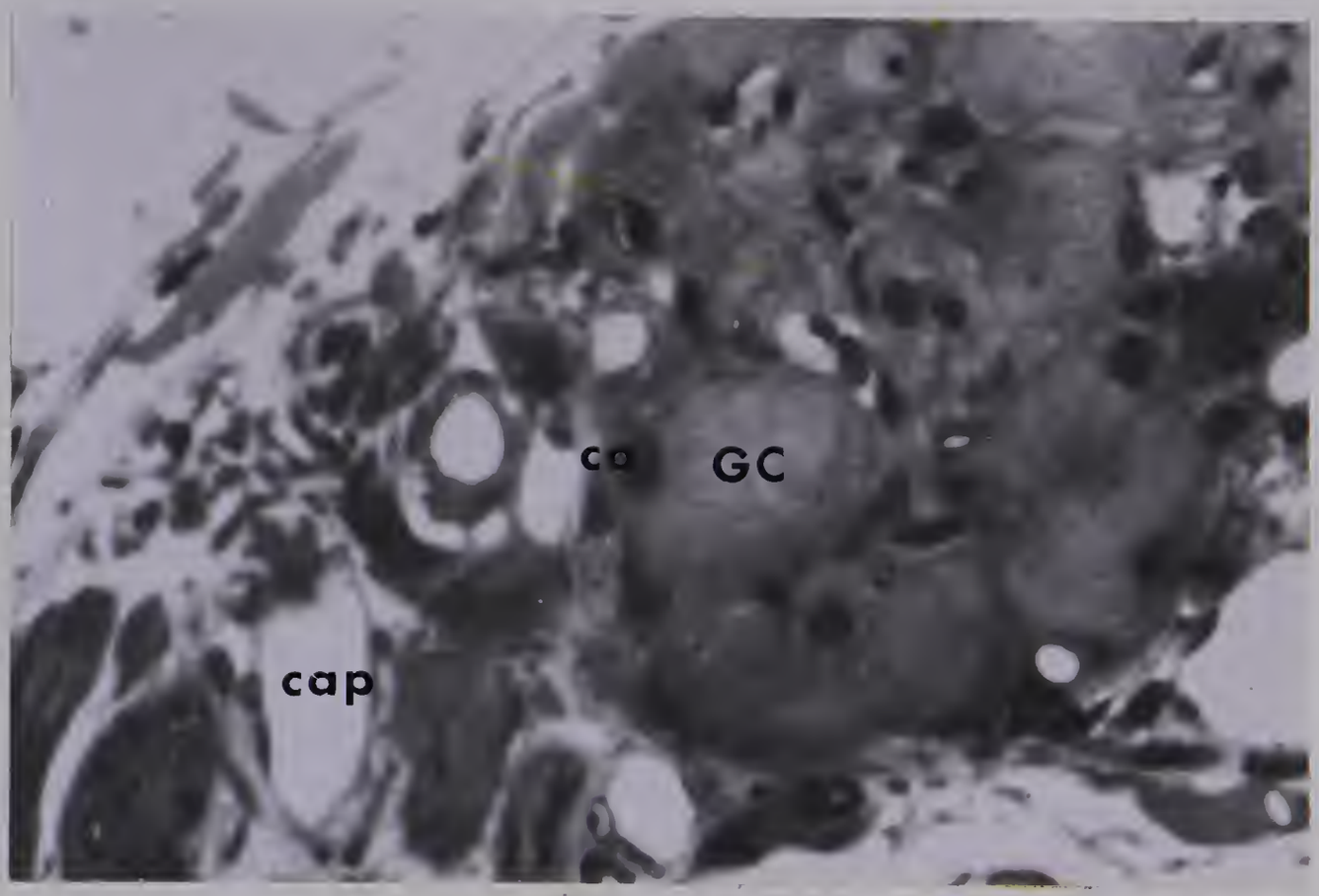
Figure 4. A photomicrograph of an Epon section of a small cardiac ganglion, stained with Richardsons stain. Nuclei of capsule cells (ca) surrounding individual ganglion cells (GC) stain darkly. There are a number of capillaries (cap) in the region of the ganglion.

Richardsons stain. Magnification x 2,000.

Figure 5. A photomicrograph of a single ganglion cell (GC) stained with thionine. Mitochondria (m) are visible as dark bodies in the cell cytoplasm. The elongated dark staining nucleus of a capsule cell (ca) can be seen close to a ganglion cell body. The pale nucleus (N) and dark staining nucleolus (Nu) of the ganglion cells can be clearly seen.

Richardsons stain. Magnification x 3,000.



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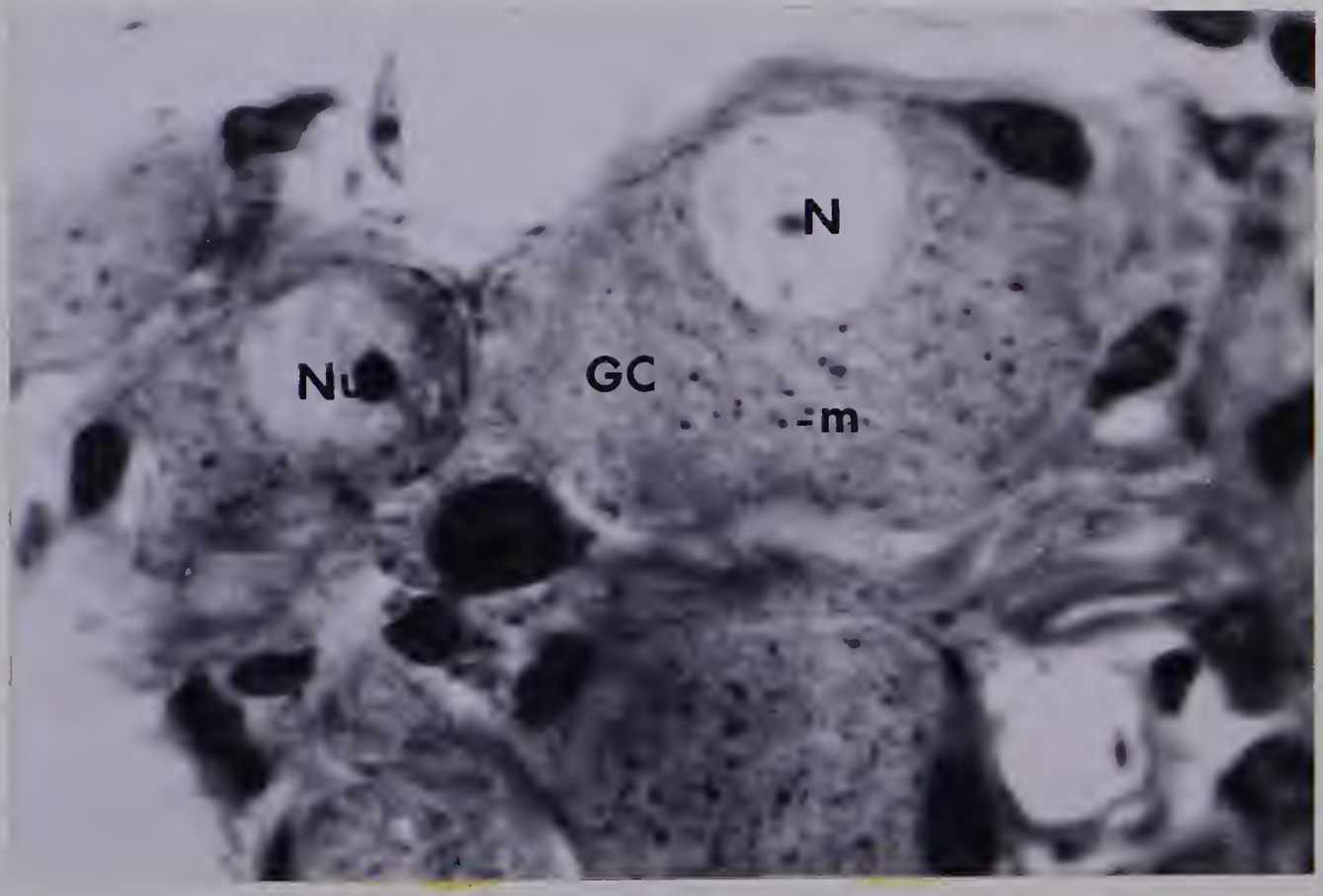

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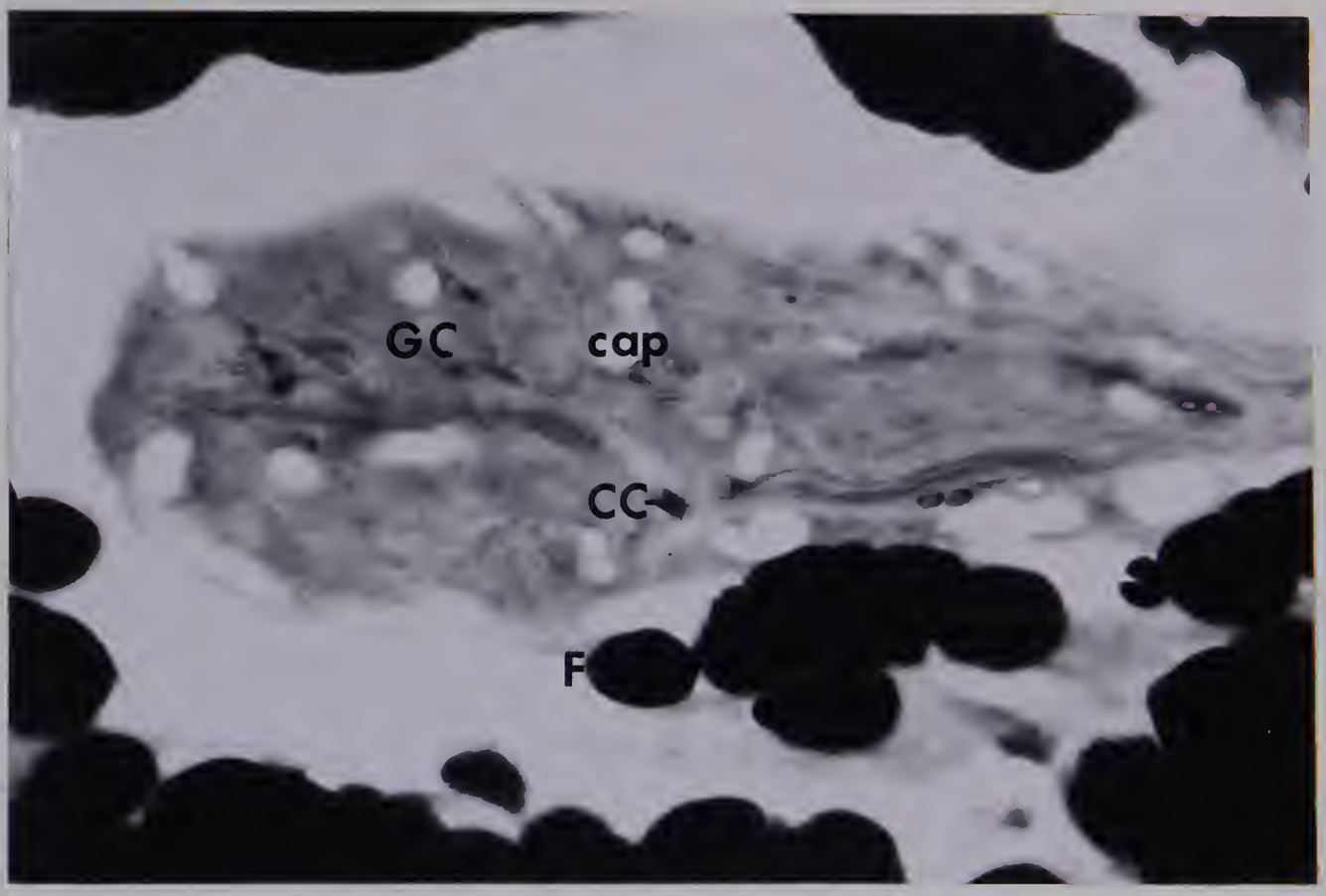
Figure 6. A photomicrograph of an Epon section of a glutaraldehyde fixed group of ganglion cells (GC) showing many capillaries (cap) throughout the ganglion and also dark staining catecholamine-containing cells (CC). Fat cells (F) are seen surrounding the ganglion.

Osmium tetroxide stain. Magnification x 300.

Figure 7. A photomicrograph of an Epon section of a glutaraldehyde fixed group of ganglion cells showing many capillaries (cap) throughout the ganglion and also dark staining catecholamine-containing cells (CC).

Osmium tetroxide stain. Magnification x 300.



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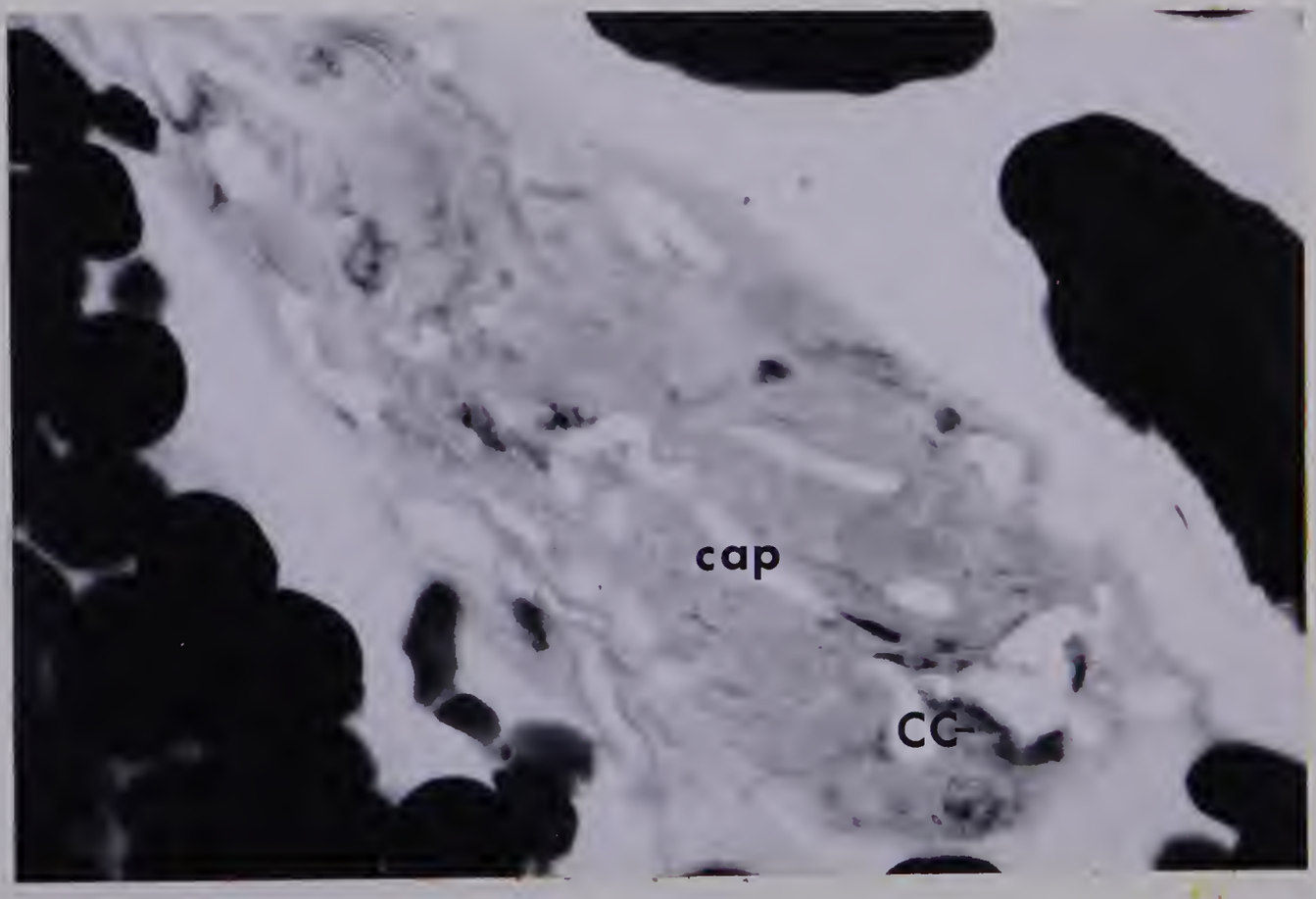

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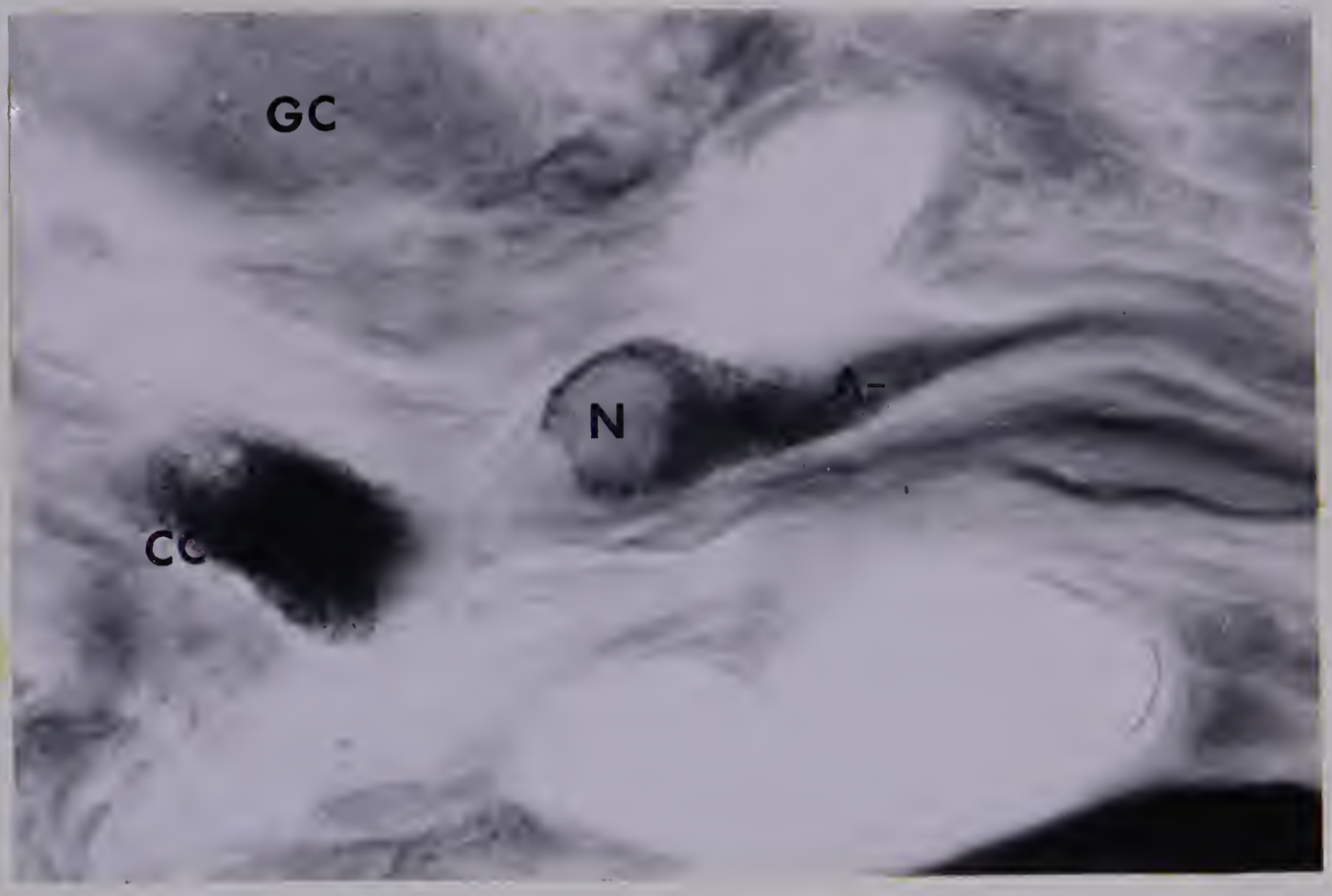
Figure 8. A photomicrograph of a catecholamine-containing cell (CC) with a long process (A) (detail of Fig. 6). The pale nucleus (N) of this cell can be clearly seen. A ganglion cell is situated close to the catecholamine-containing cells.

Osmium tetroxide stain. Magnification x 3,000.

Figure 9. A photomicrograph of catecholamine-containing cells (CC) near capillaries (cap) in a cardiac ganglion.

Osmium tetroxide stain. Magnification x 3,000.



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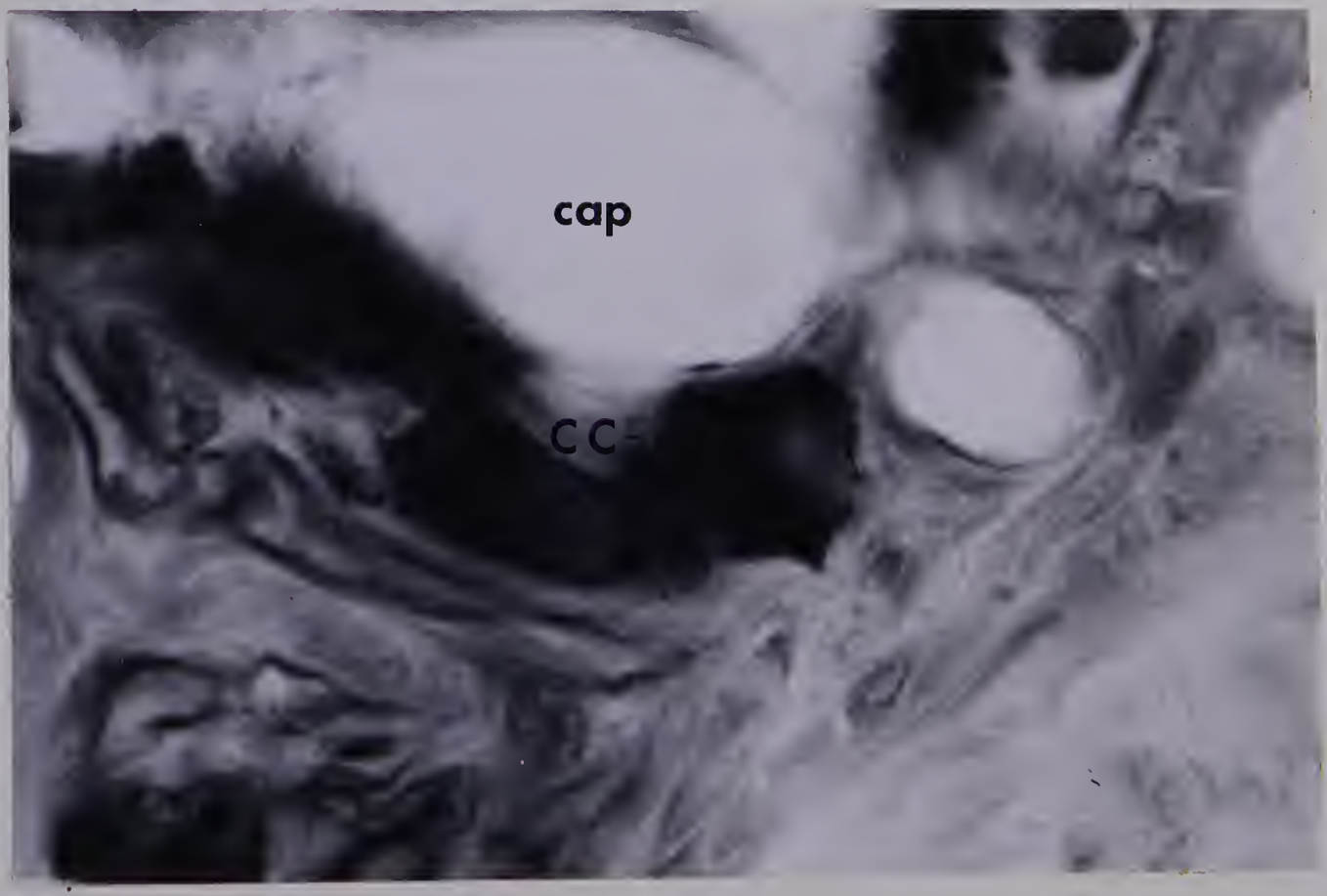

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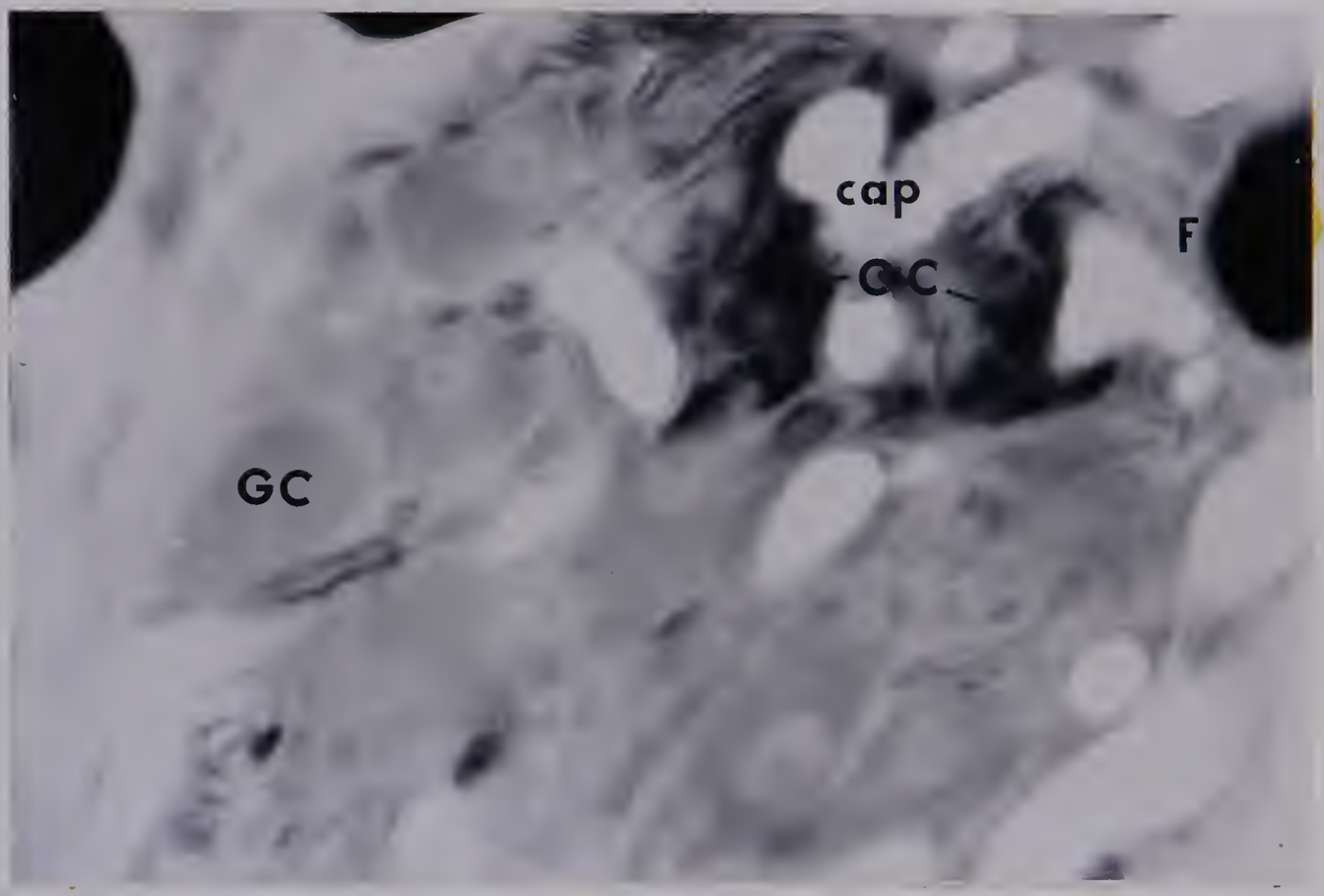
Figure 10. A photomicrograph of a group of ganglion cells (GC) and catecholamine-containing cells (CC). The latter are clustered around capillaries (cap). Several large dark staining fat cells can be seen.

Osmium tetroxide stain. Magnification x 800.

Figure 11. A photomicrograph of a catecholamine-containing cell (CC) in a ganglion situated some distance from a capillary (cap).

Osmium tetroxide stain. Magnification x 800.



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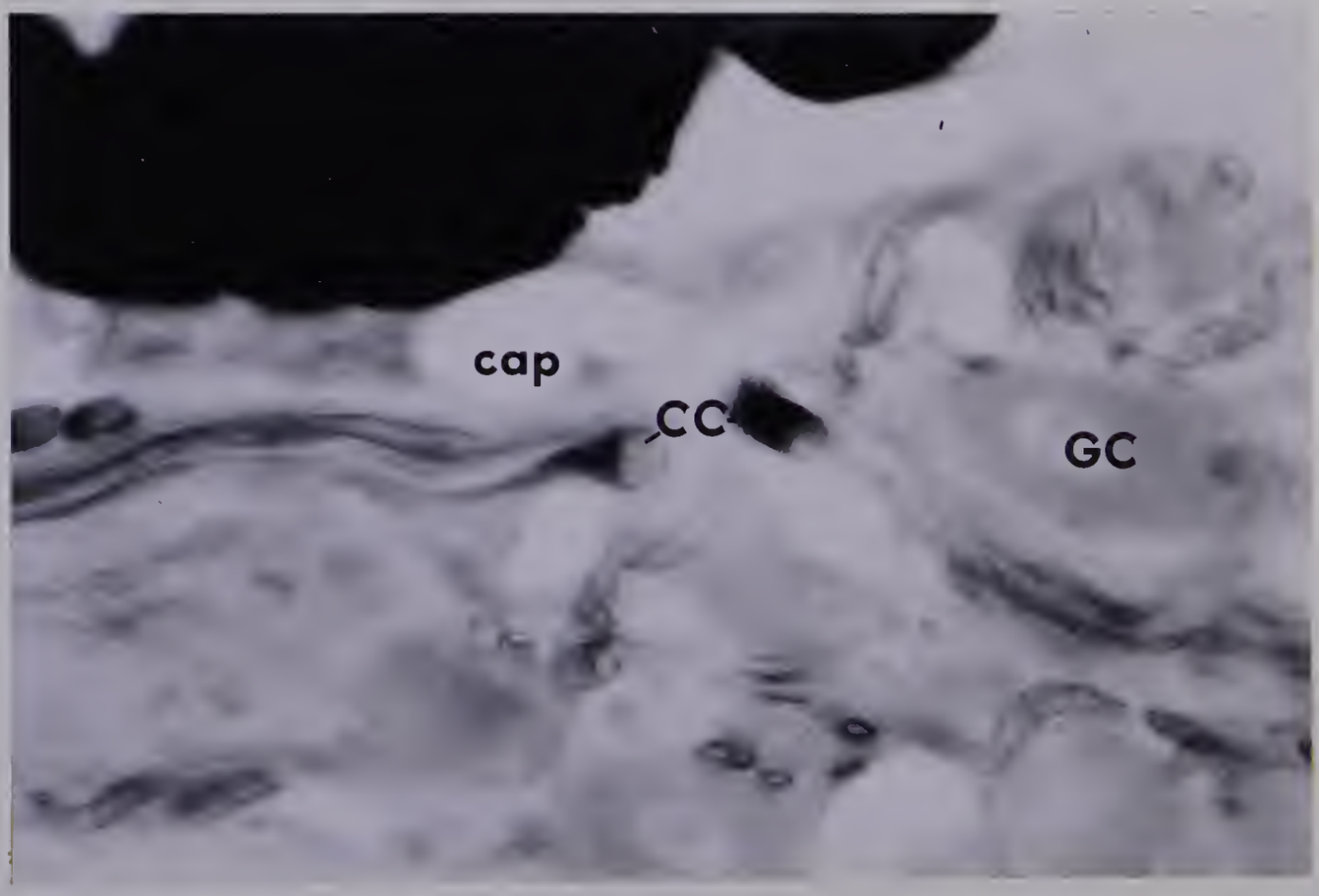


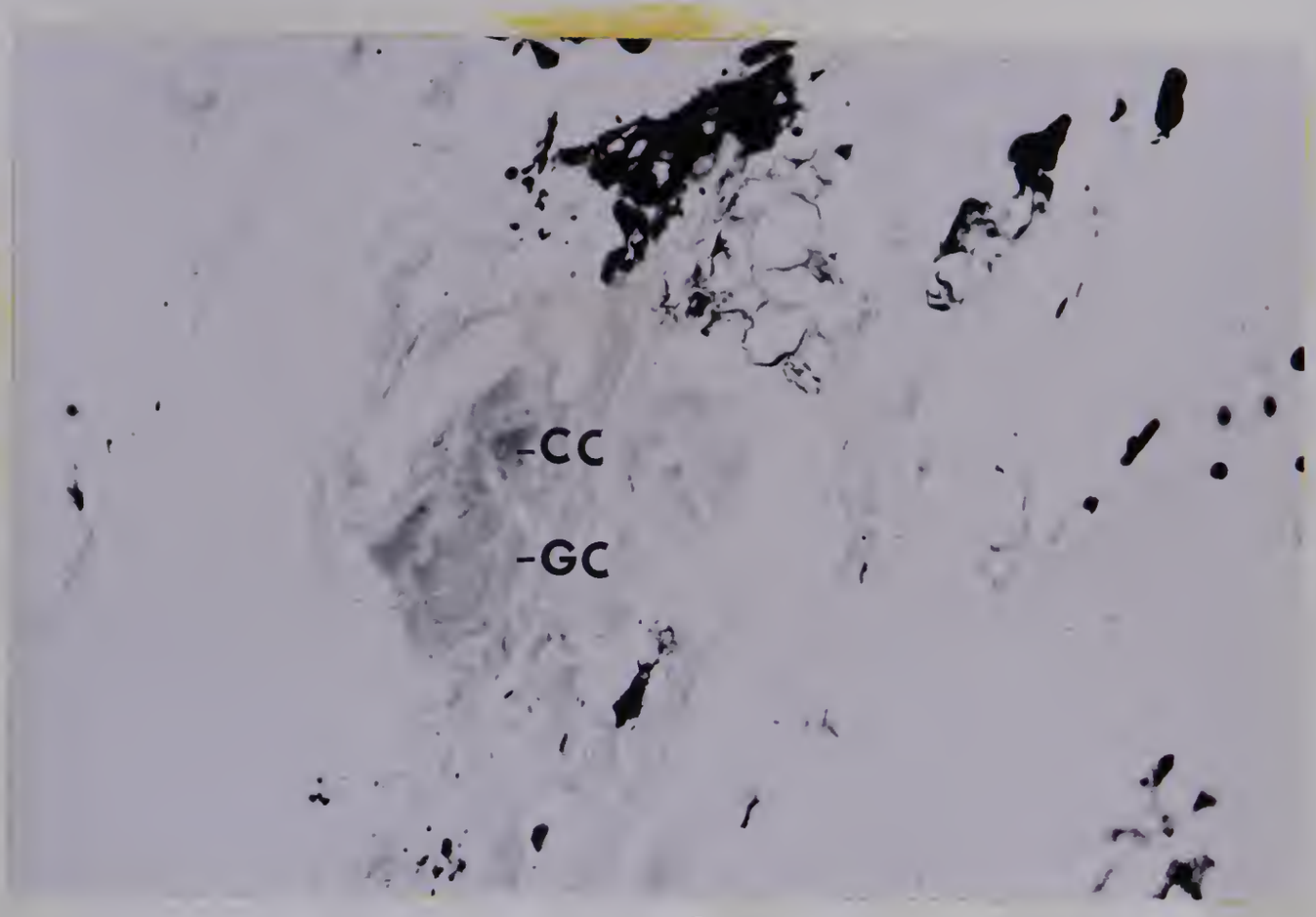
11

Figure 12. A photomicrograph of glutaraldehyde and osmium tetroxide treated paraffin section of cat heart tissue. Note ganglion cell (GC) and dark appearing catecholamine-containing cells (CC).

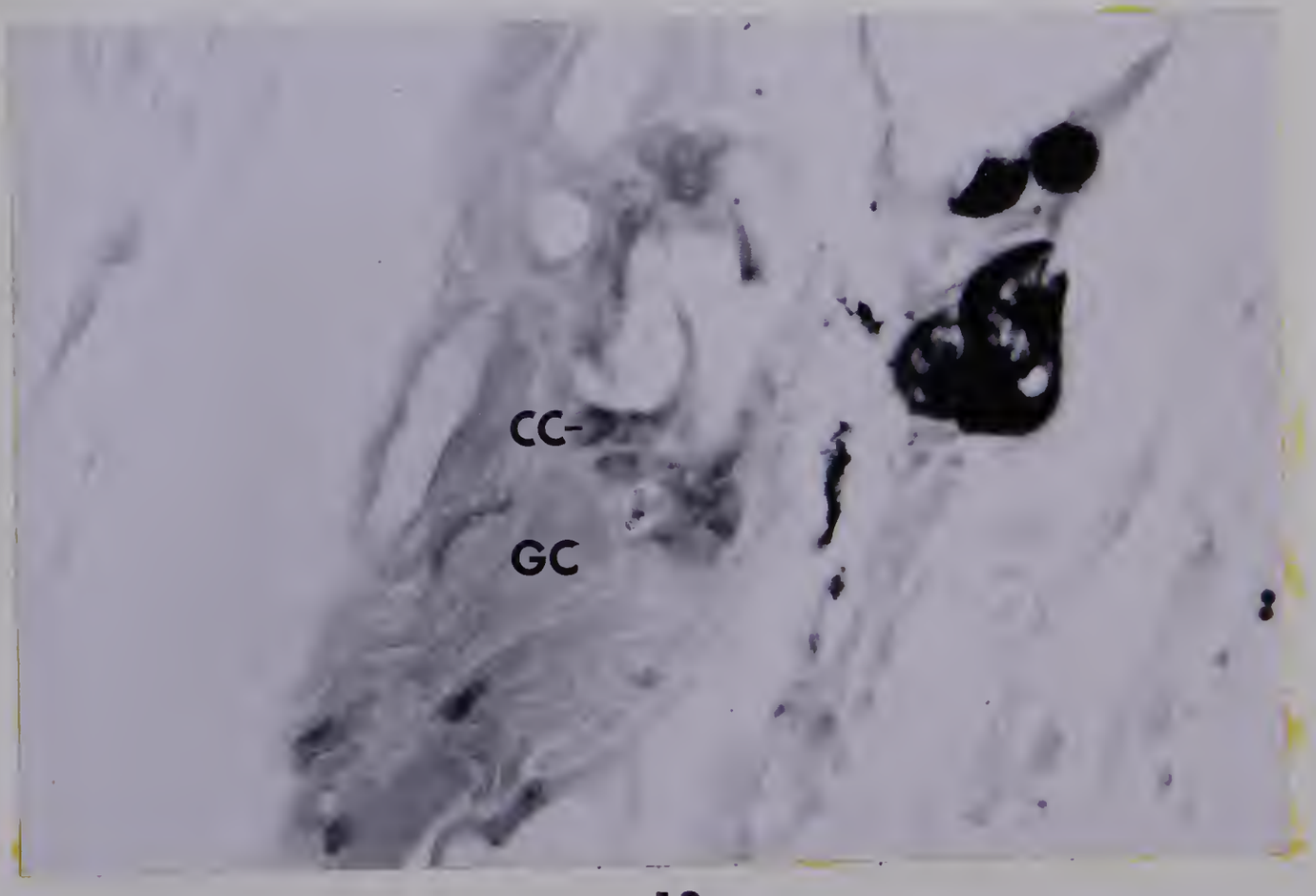
Osmium tetroxide stain. Magnification x 200.

Figure 13. A photomicrograph at a higher magnification of a similar section as shown in Figure 12. A dark stained catecholamine-containing cell (CC) can be seen near a capillary. A ganglion cell (GC) is situated below it.

Osmium tetroxide stain. Magnification x 800.



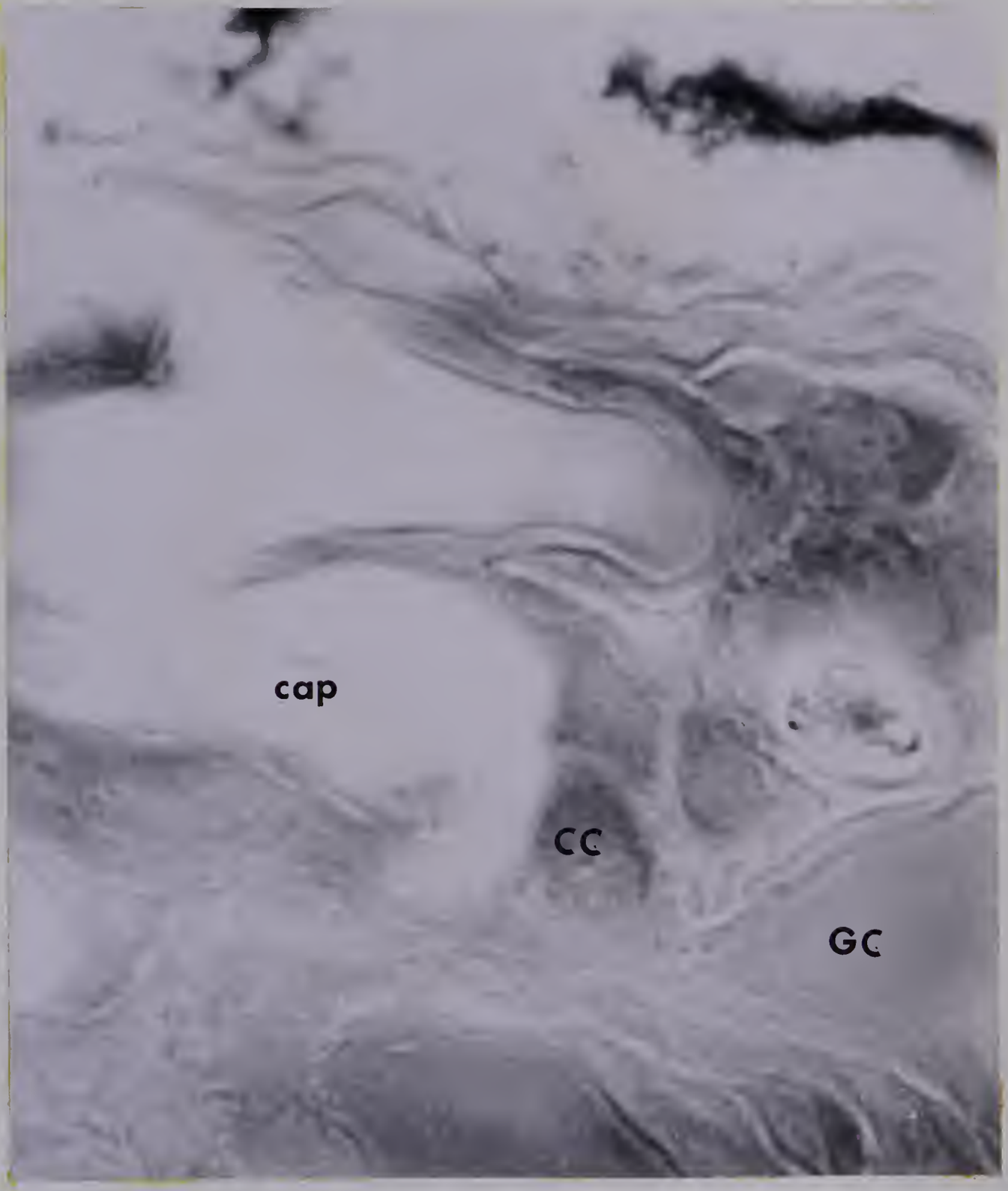
12



13

Figure 14. A photomicrograph showing an enlargement of the cells marked in Figure 13. Dark appearing catecholamine-containing cells (CC) are near a capillary (cap). A lighter staining ganglion cell (GC) can also be seen.

Osmium tetroxide stain. Magnification x 3,200.



14

Figure 15. A low power electronmicrograph of two cardiac ganglion cells. The large central nucleus and nucleolus of one ganglion cell can be seen. A satellite or Schwann cell can be seen near the ganglion cells. Nerve fibers surrounded by collagen are seen in cross section near the ganglion cells. A fibroblast is located near the peripheral edge of the ganglion.

Ganglion cell	GC
Nucleus	N
Nucleolus	Nu
Satellite cell	Sc
Collagen	C
Nerve fiber	NF
Cytoplasm	Cy
Fibroblast	F

Lead citrate and uranyl acetate stain.

Magnification x 6,600.

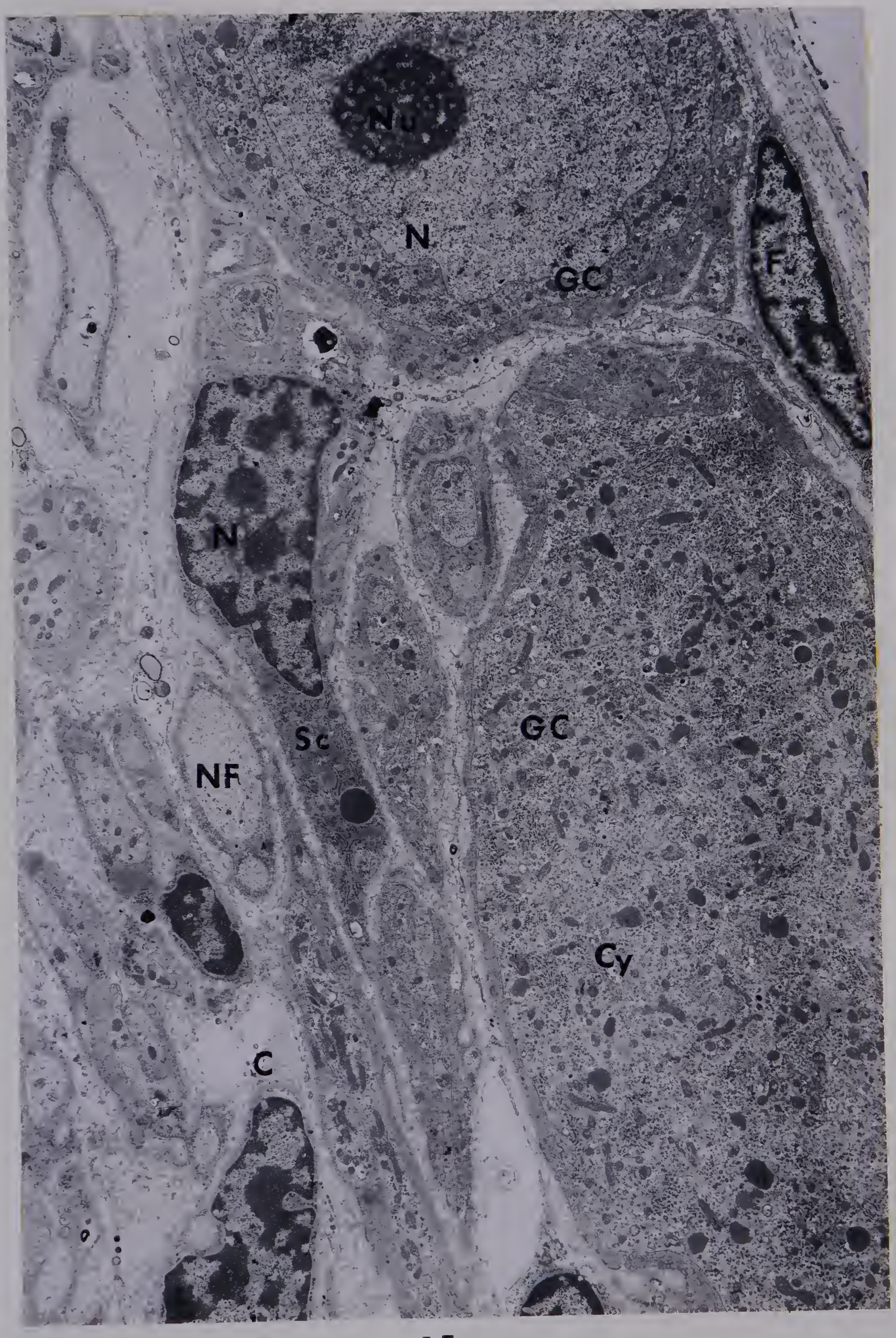
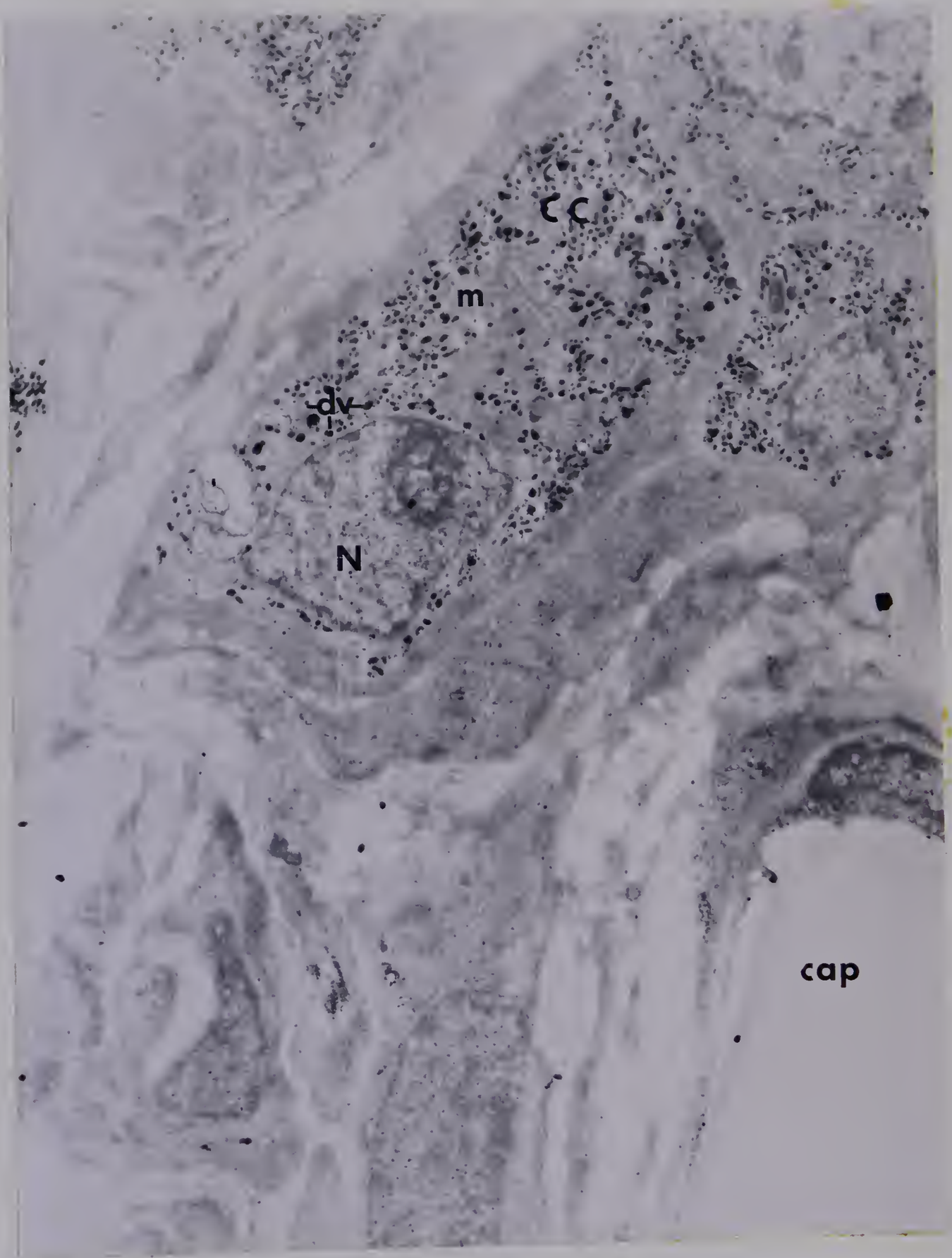

Nu
N
GC
F
N
Sc
GC
NF
Cy
C

Figure 16. An electronmicrograph showing catecholamine-containing cells (chromaffin-type) in a group near a capillary. These cells are contained within a cardiac ganglion.

Chromaffin-type cell	CC
Nucleus	N
Mitochondria	m
Capillary	cap
Dark-cored vesicles	dv

Lead citrate and uranyl acetate stain.

Magnification x 9,000.



16

Figure 17. An electronmicrograph of the details of cardiac ganglion cell cytoplasm showing a Golgi body, lysosomes, mitochondria, neurotubules, a multivesicular body, and endoplasmic reticulum.

Golgi body	g
Lysosome	l
Mitochondria	m
Neurotubules	n
Multivesicular body	mv
Endoplasmic reticulum	er

Lead citrate and uranyl acetate stain.

Magnification x 33,000.

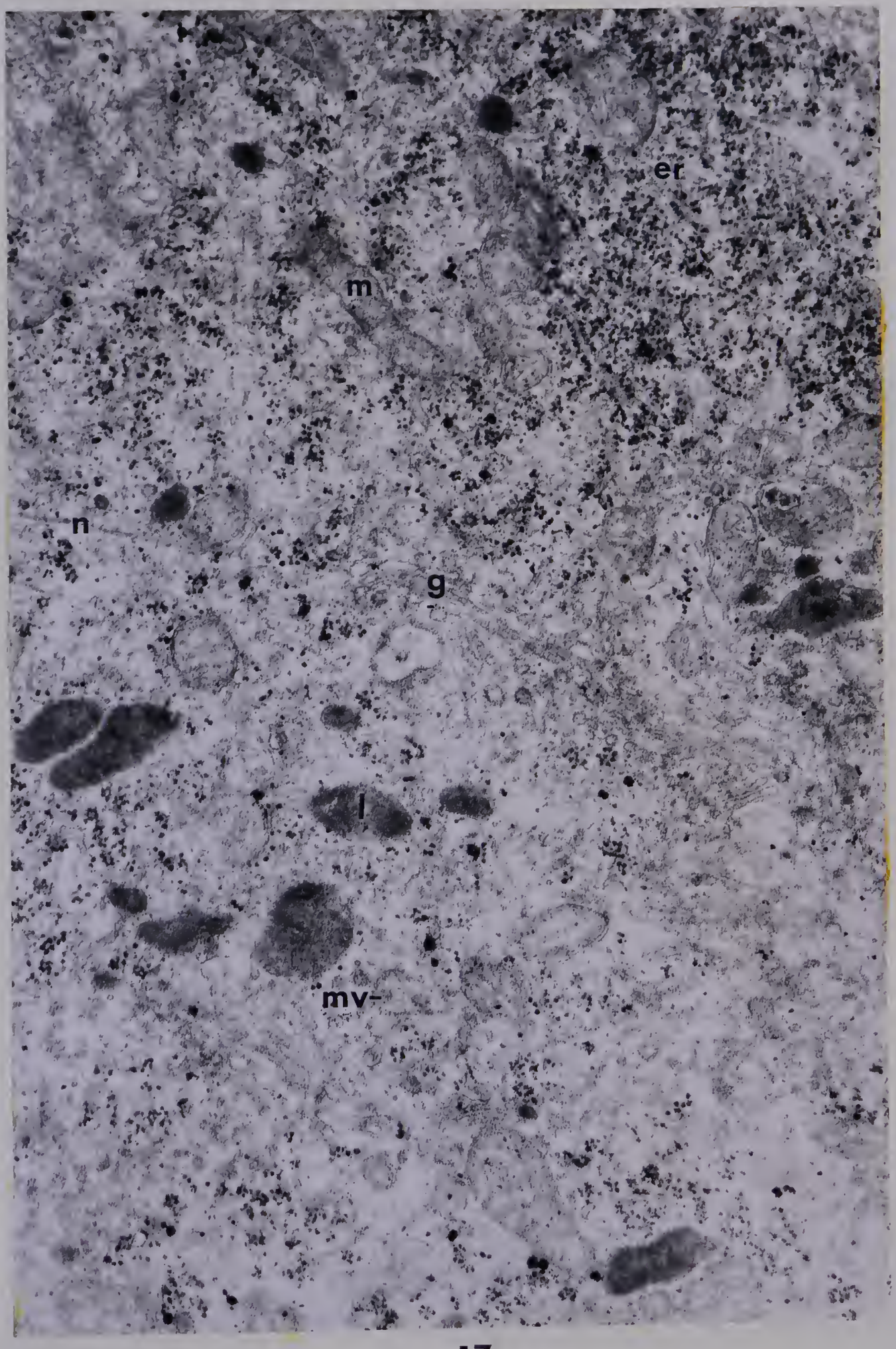

er
m
n
g
l
mv

Figure 18. An electronmicrograph of a process leaving a ganglion cell showing details of cytoplasm and neurotubules where the dendrite leaves the soma. Vesicle-containing nerve endings and small processes are seen near the ganglion cell and near the large process leaving it.

Ganglion cell	GC
Process	P
Neurotubules	n
Nerve endings	Ne
Small process	Sp

Lead citrate and uranyl acetate stain.

Magnification x 13,500.

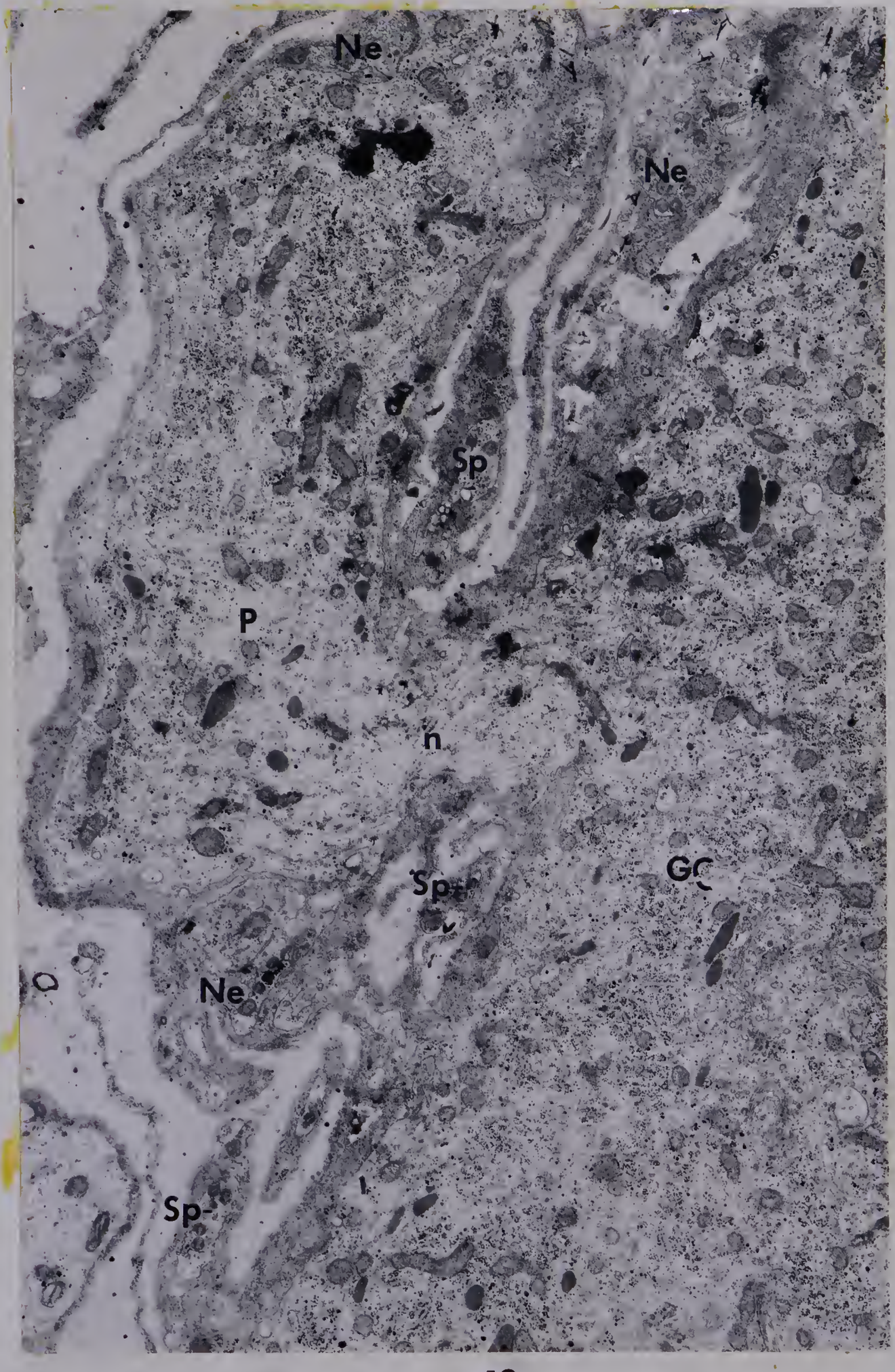

Ne
Ne
Sp
P
n
Sp
GC
Ne
Sp

Figure 19. An electronmicrograph of a cross section of a dendrite showing a glycogen membrane complex in concentric arrangement.

Dendrite	A
Glycogen membrane complex	Gl

Lead citrate and uranyl acetate stain.

Magnification x 12,800.

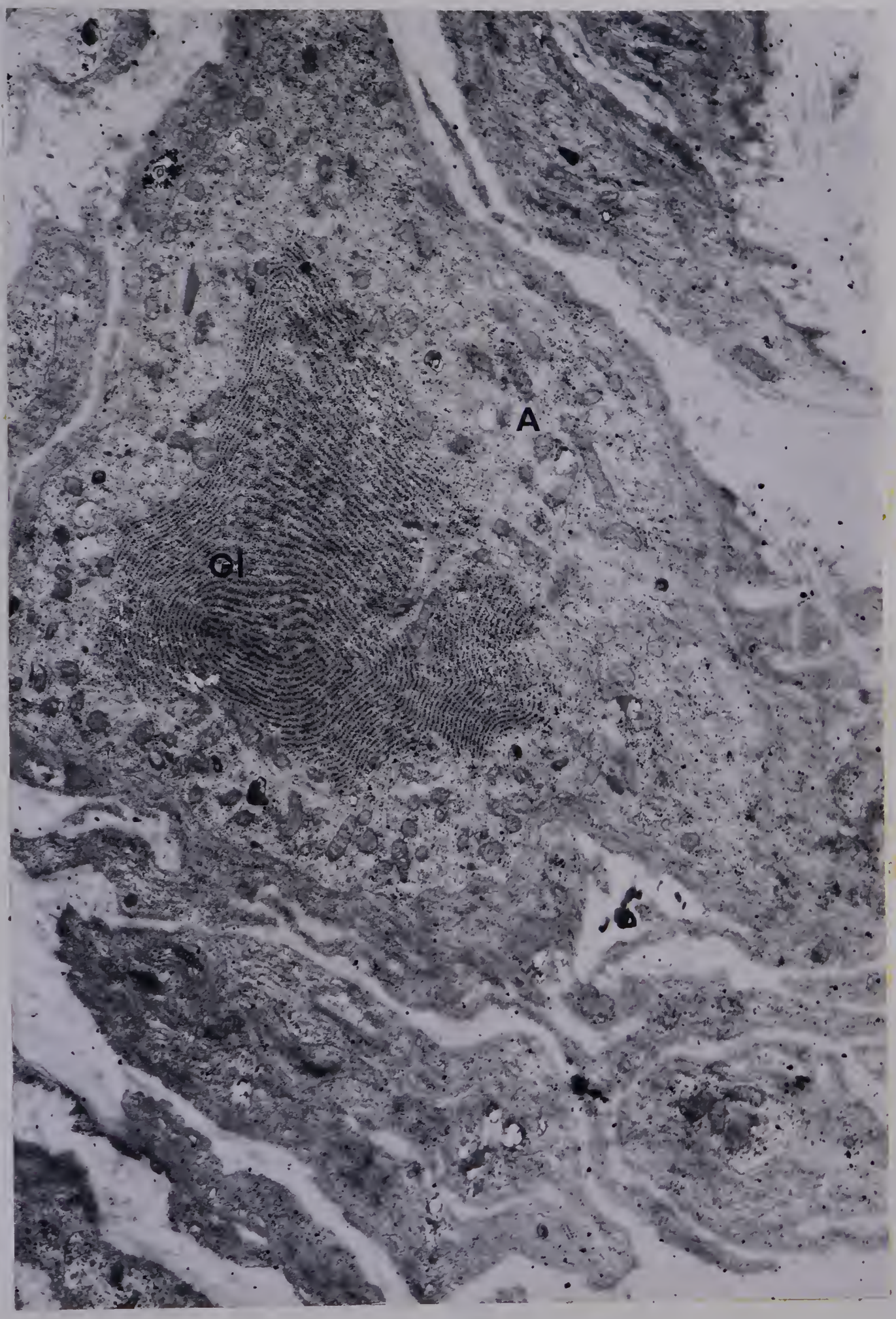

A
Gl

Figure 20. An electronmicrograph showing an enlargement of an area shown in Figure 19. The close proximity of mitochondria to the complex can be seen. The arrangement of the granules and membrane layers is more clearly shown.

Mitochondria	m
Granules	g
Membrane layers	M

Lead citrate and uranyl acetate stain.

Magnification × 30,000.

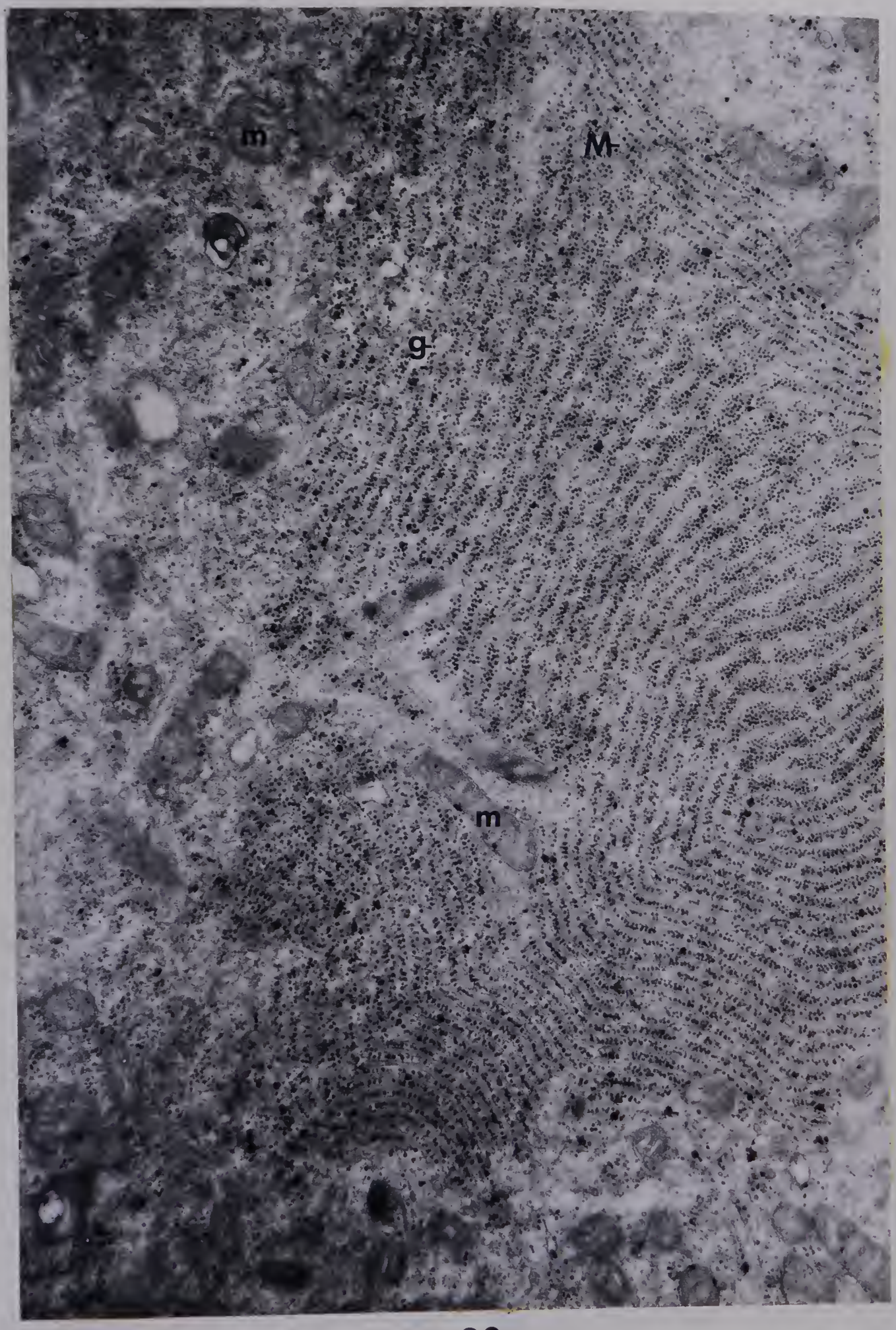

m
M
g
m

Figure 21. An electronmicrograph at high magnification of a flat array of glycogen and membrane complex as shown in Figures 19 and 20.

Mitochondria	m
Membrane	M
Glycogen granules	g

Lead citrate and uranyl acetate st in.

Magnification x 96,000.

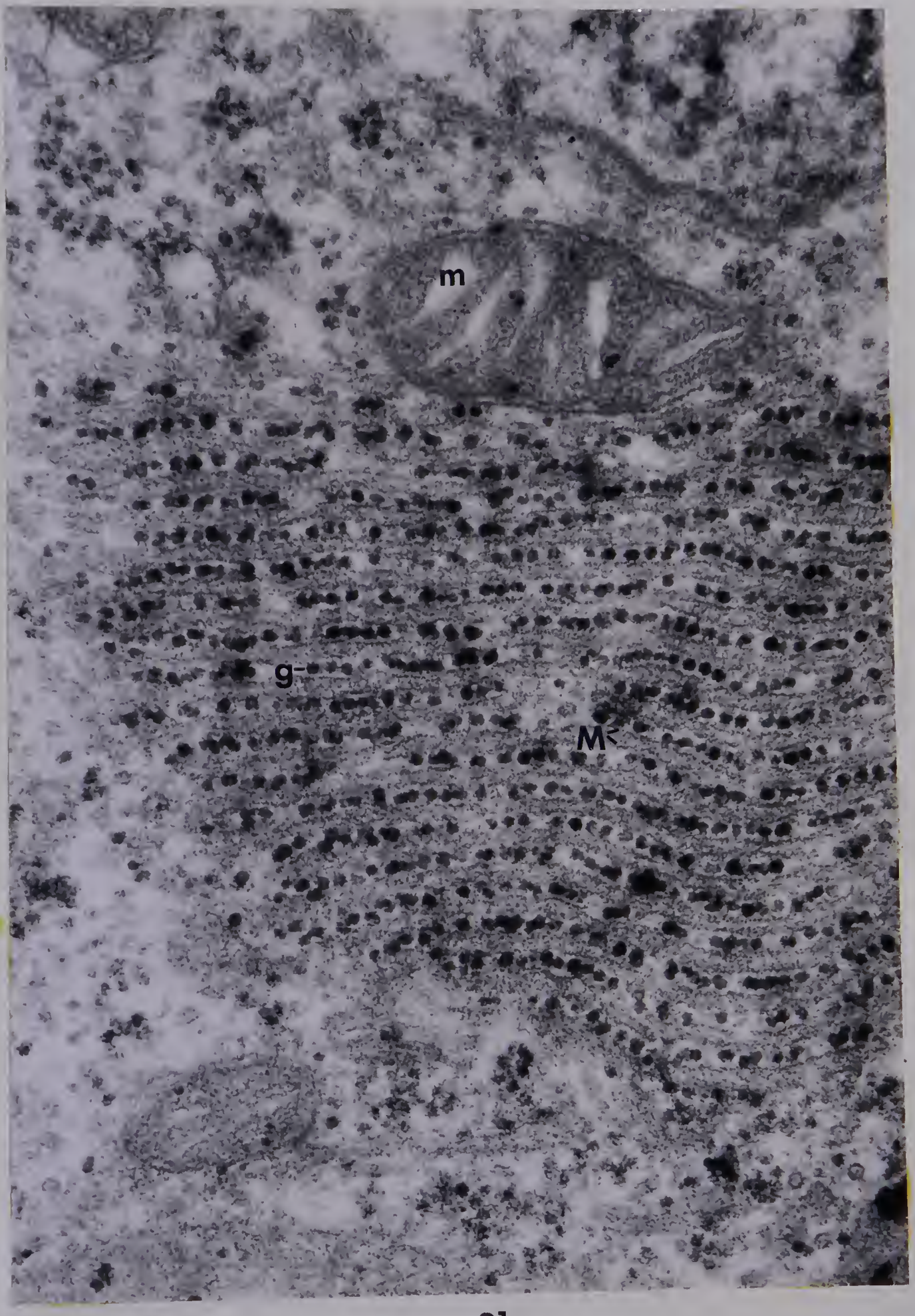

m
g
M

Figure 22. An electronmicrograph of unmyelinated axons embedded in Schwann cell cytoplasm, with the mesaxon visible. The smaller axon is synapsing with the larger one.

Schwann cell	Sch
Nucleus	N
Axon	A
Mitochondria	m
Synapse	S
Synaptic vesicles	SV
Mesaxon	mes

Lead citrate and uranyl acetate stain.

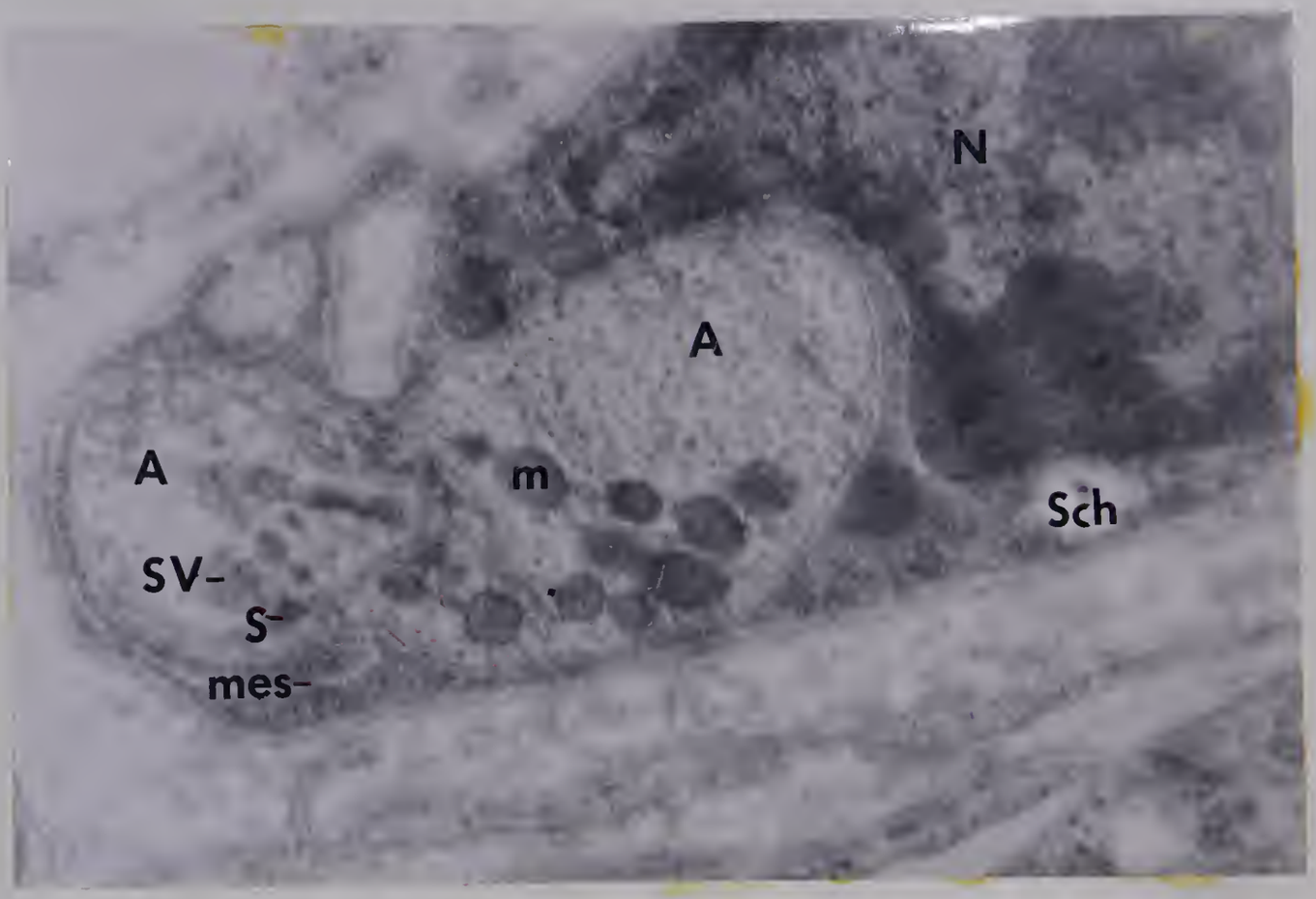
Magnification x 37,500.

Figure 23. An electronmicrograph of a group of unmyelinated axons, one myelinated axon, collagen and nucleus of the Schwann cell which is the sheath of the nerve bundle.

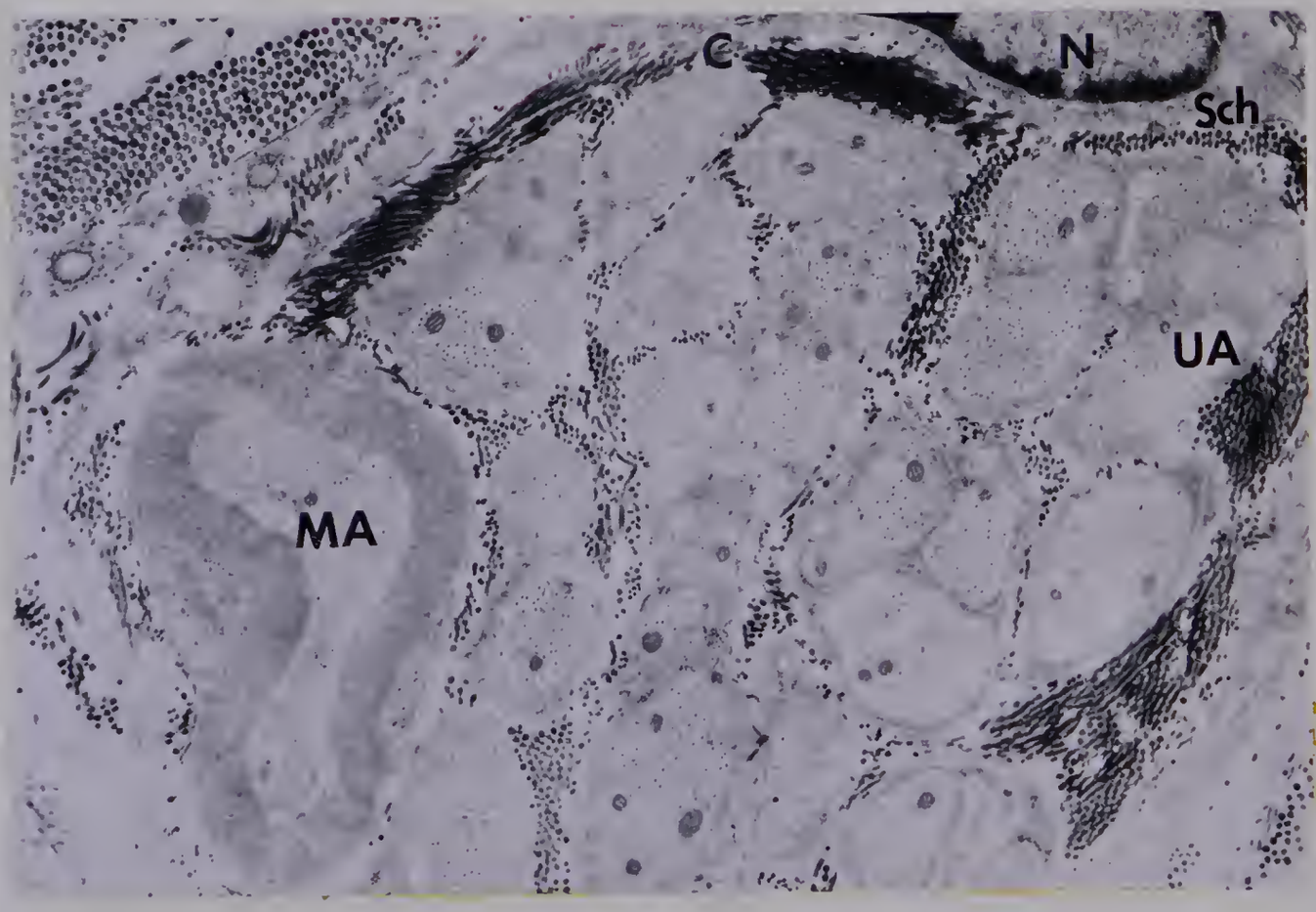
Schwann cell	Sch
Nucleus	N
Collagen	C
Unmyelinated axons	UA
Myelinated axons	MA

Lead citrate and uranyl acetate stain.

Magnification x 22,500.



22



23

Figure 24. An electromicrograph of a myelinated axon with Schwann cell nucleus.

Schwann cell	Sch
Nucleus	N
Myelinated axon	MA

Lead citrate and uranyl acetate stain.

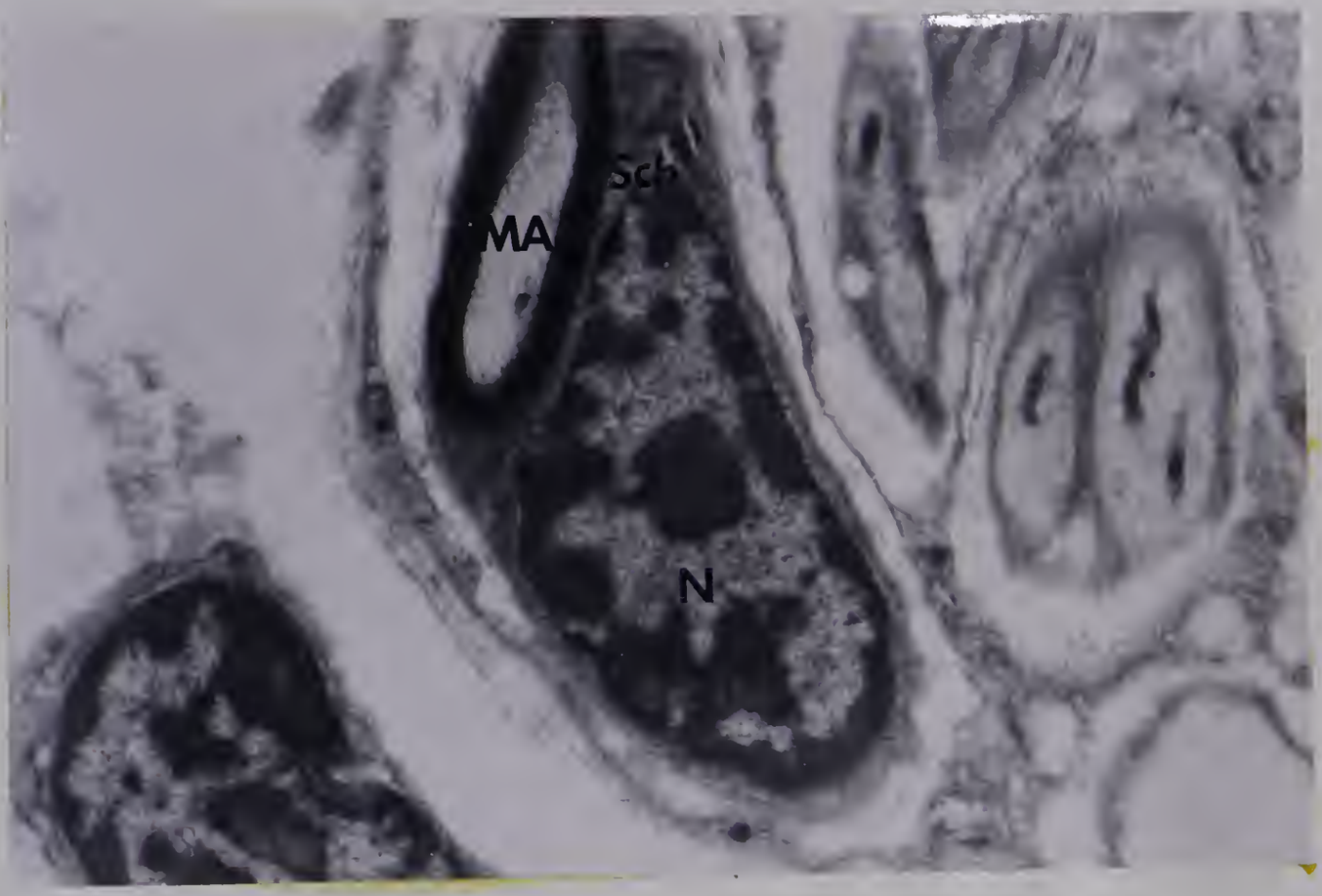
Magnification x 8,800.

Figure 25. An electromicrograph of the edge of a ganglion cell, showing a satellite cell nucleus and layers of satellite cell cytoplasm around the edge of the ganglion cell.

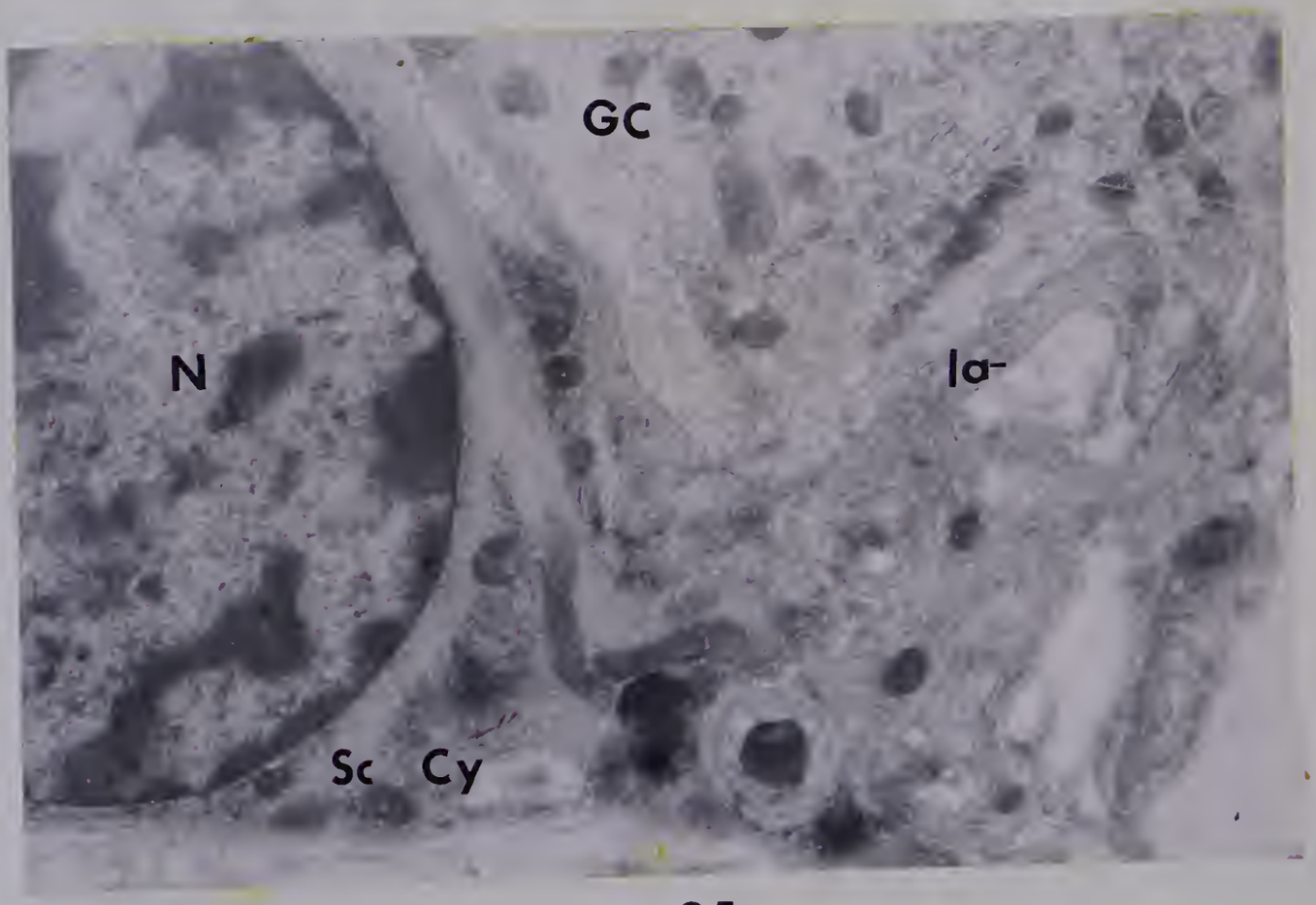
Ganglion cell	GC
Satellite cell	Sc
Nucleus	N
Cytoplasm	Cy
Layers of cytoplasm	la

Lead citrate and uranyl acetate stain.

Magnification x 17,000.



24



25

Figure 26. An electronmicrograph of layers of satellite cell cytoplasm around the edge of the ganglion cell showing finger-like projections of the layers surrounded by the basement membrane. Collagen can be seen between the projections.

Ganglion cell	GC
Layers of cytoplasm	la
Projections	P
Collagen	C
Basement membrane	BM

Lead citrate and uranyl acetate stain.

Magnification × 29,000.

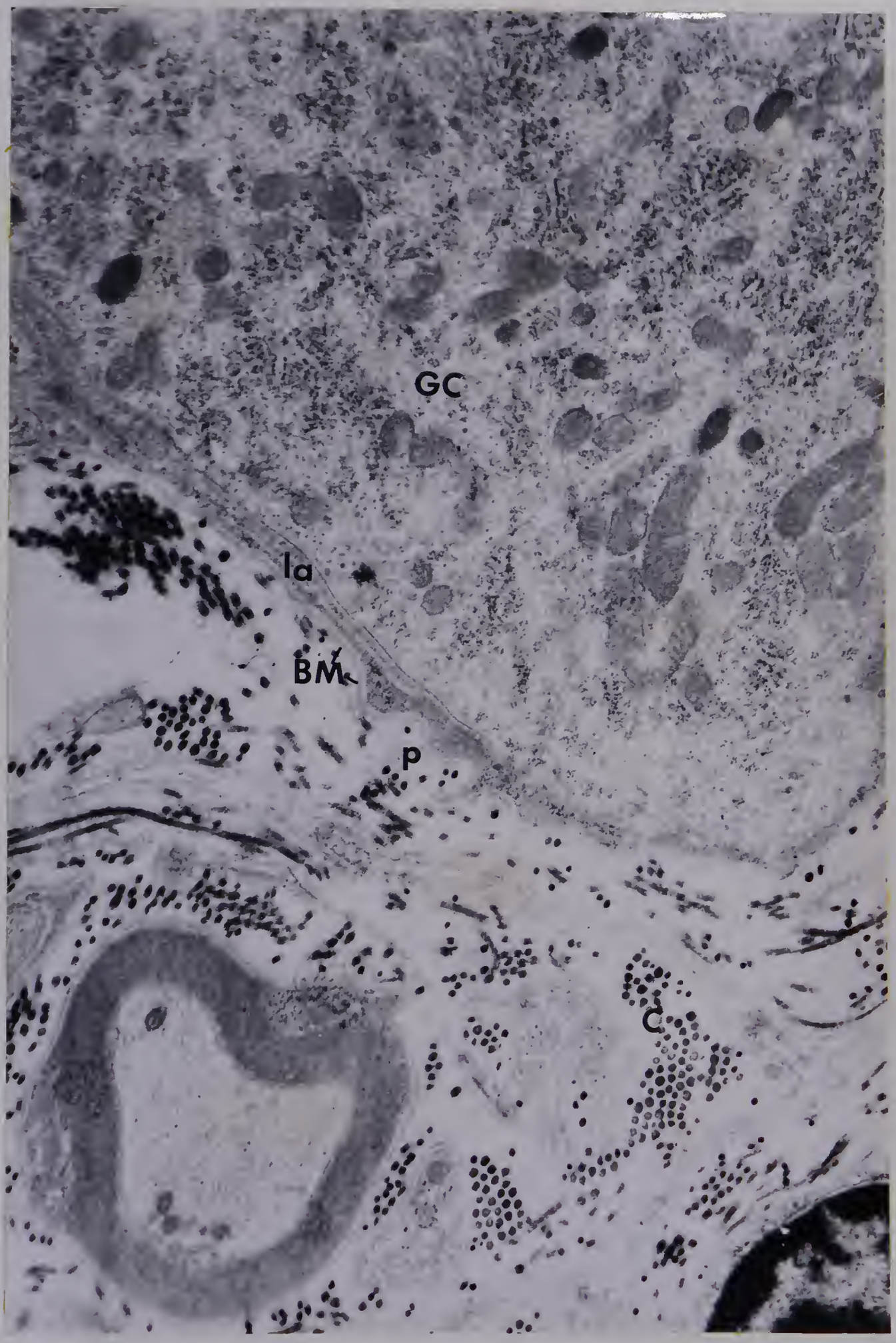

GC
la
BM
p
C

Figure 27. An electronmicrograph of layers of satellite cell cytoplasm surrounding a vesicle-filled process which is synapsing with a smaller process.

Layers of cytoplasm	la
Process	p
Synaptic vesicles	SV
Synapse	S

Lead citrate and uranyl acetate stain.

Magnification x 48,000.

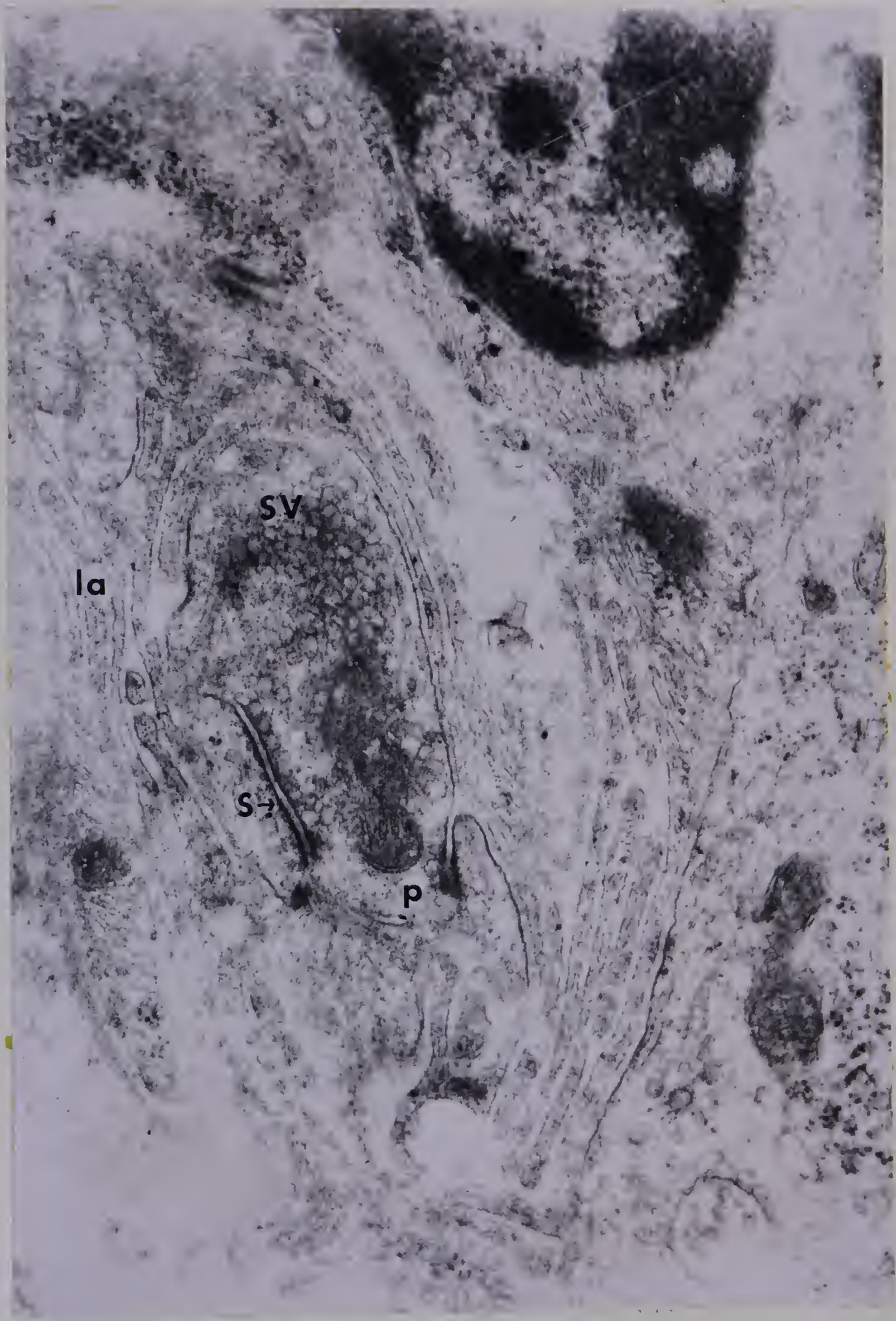

SV
Ia
S
p

Figure 28. A low power electronmicrograph of cardiac ganglion cells and a chromaffin-type cell.

Ganglion cell	GC
Chromaffin-type cell	CC

Lead citrate and uranyl acetate stain.

Magnification x 3,300.

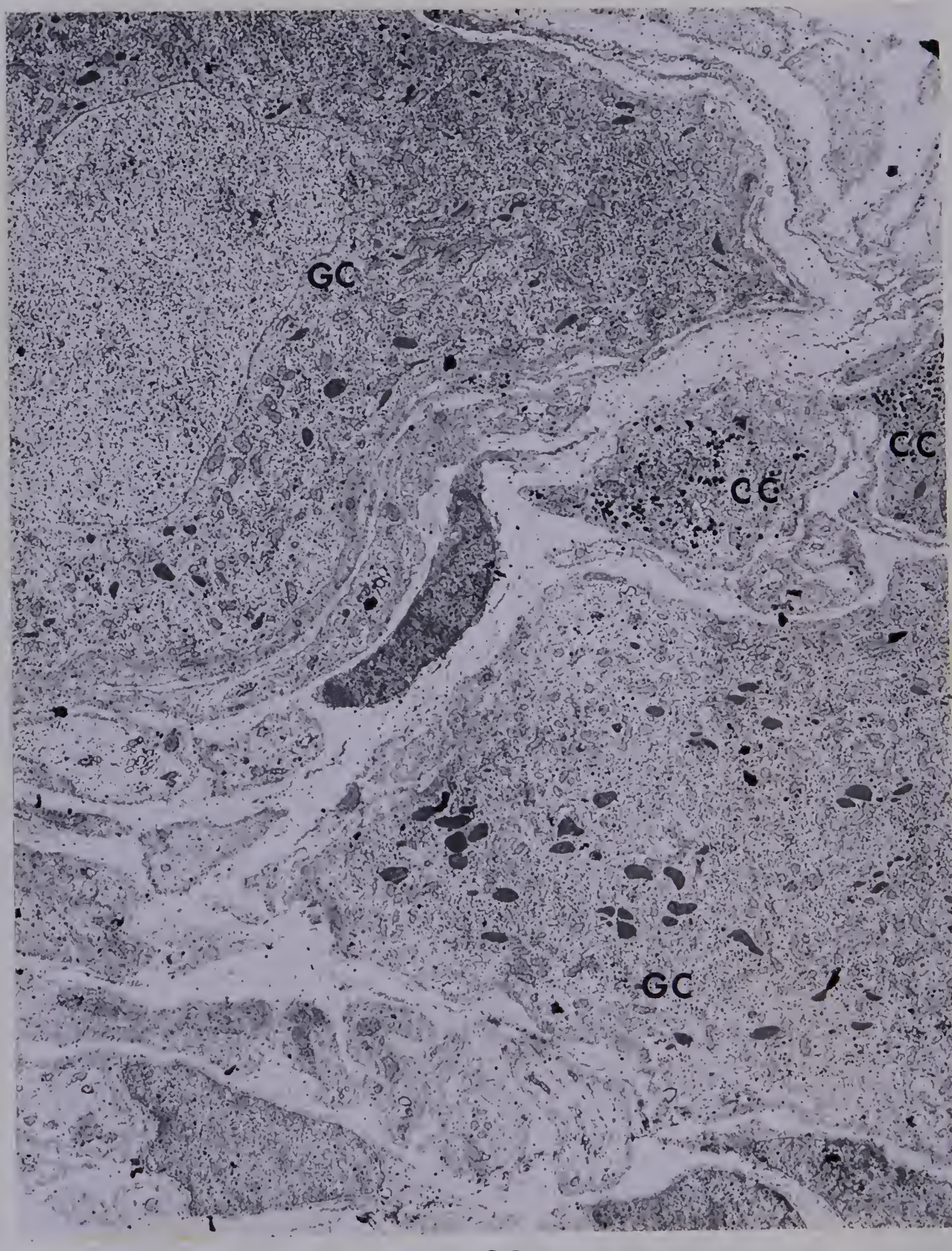

GC
CC
CC
GC

Figure 29. An electronmicrograph of a catecholamine-containing (chromaffin-type) cell showing large nucleus, dark-staining granules, mitochondria, endoplasmic reticulum and satellite cell nucleus near the catecholamine-containing cell. Also a large process containing many agranular vesicles can be seen appearing to synapse with the catecholamine-containing cells.

Chromaffin-type cell	CC
Nucleus	N
Dark granules	Dg
Mitochondria	m
Endoplasmic reticulum	er
Satellite cell nucleus	ScN
Process	P
Agranular vesicles	AgV
Collagen	C
Synapse	S

Lead citrate and uranyl acetate stain.

Magnification x 24,000.

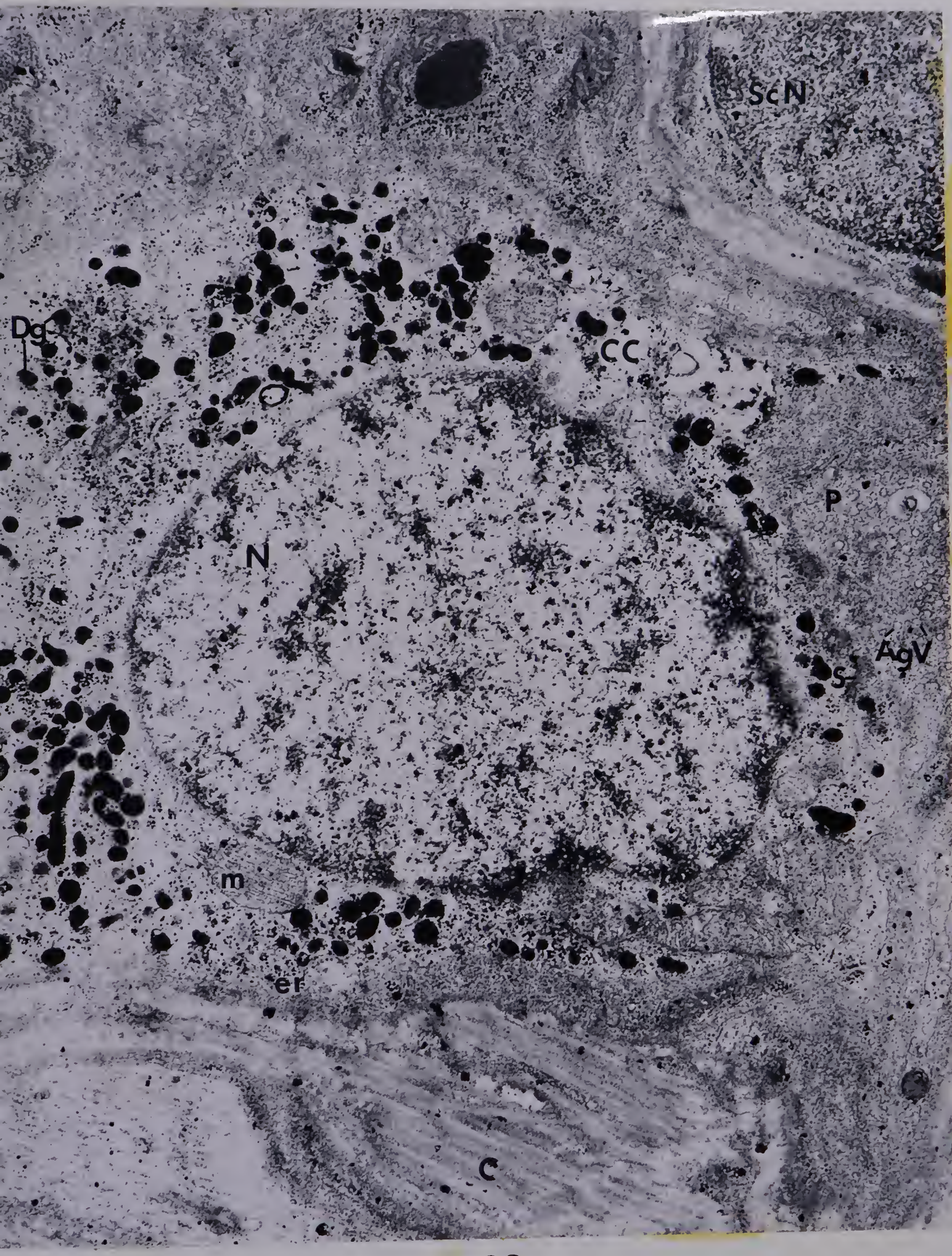

ScN
Dg
CC
P
N
AgV
S
m
er
C

Figure 30. An electronmicrograph of a process of a catecholamine-containing cell containing large dark-staining granules. Also, other processes can be seen containing large numbers of agranular vesicles near the body of the catecholamine-containing cell.

Process	P
Catecholamine-containing cell	CC
Dark granules	Dg
Agranular vesicles	AgV
Synapse	S

Lead citrate and uranyl acetate stain.

Magnification x 34,500.

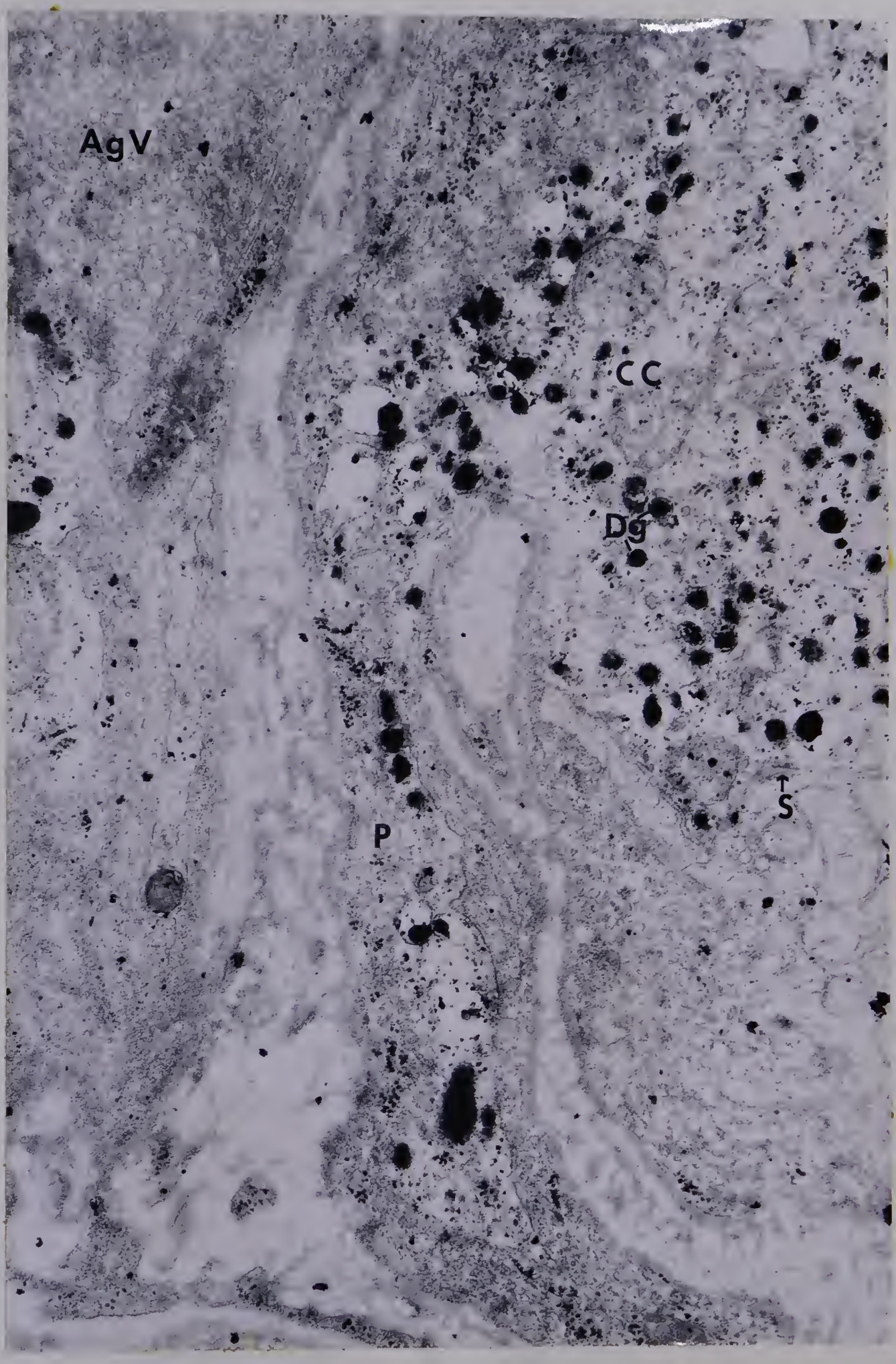

AgV
CC
Dg
S
P

Figure 31. An electronmicrograph of a catecholamine-containing process with dark granular vesicles. The vesicles are surrounded by a double membrane, and have a large, dense core, with a smaller, less dense area just inside the membrane. Units of small granules within the dark core can sometimes be seen.

Dark granules	Dg
Double membrane	dm
Dense core	Dc
Small granules	Sg

Lead citrate and uranyl acetate stain.

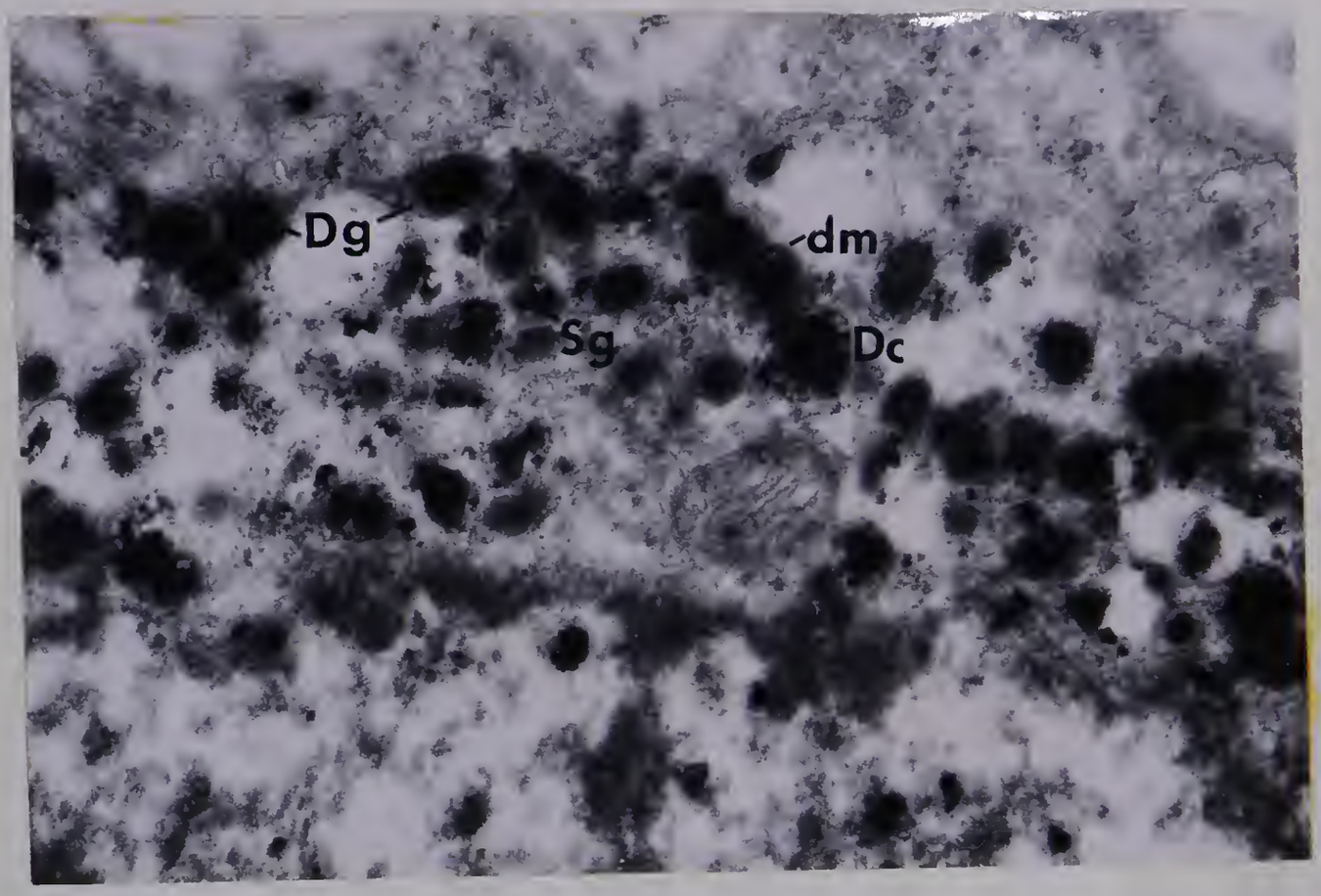
Magnification x 70,000.

Figure 32. An electronmicrograph of a nerve process containing many agranular vesicles, a mitochondrion and a few large opaque vesicles.

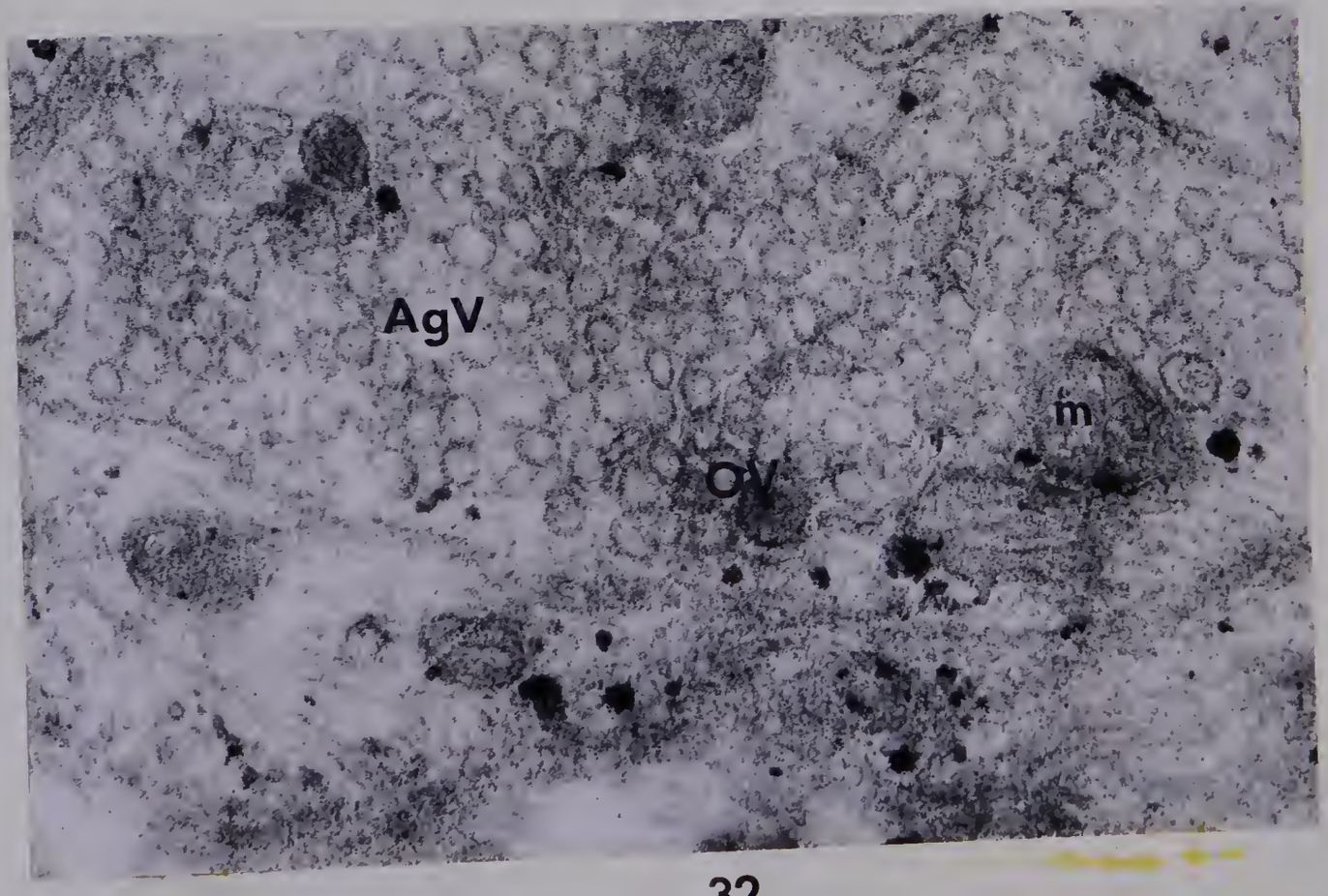
Agranular vesicles	AgV
Mitochondrion	m
Opaque vesicles	OV

Lead citrate and uranyl acetate stain.

Magnification x 150,000.



31



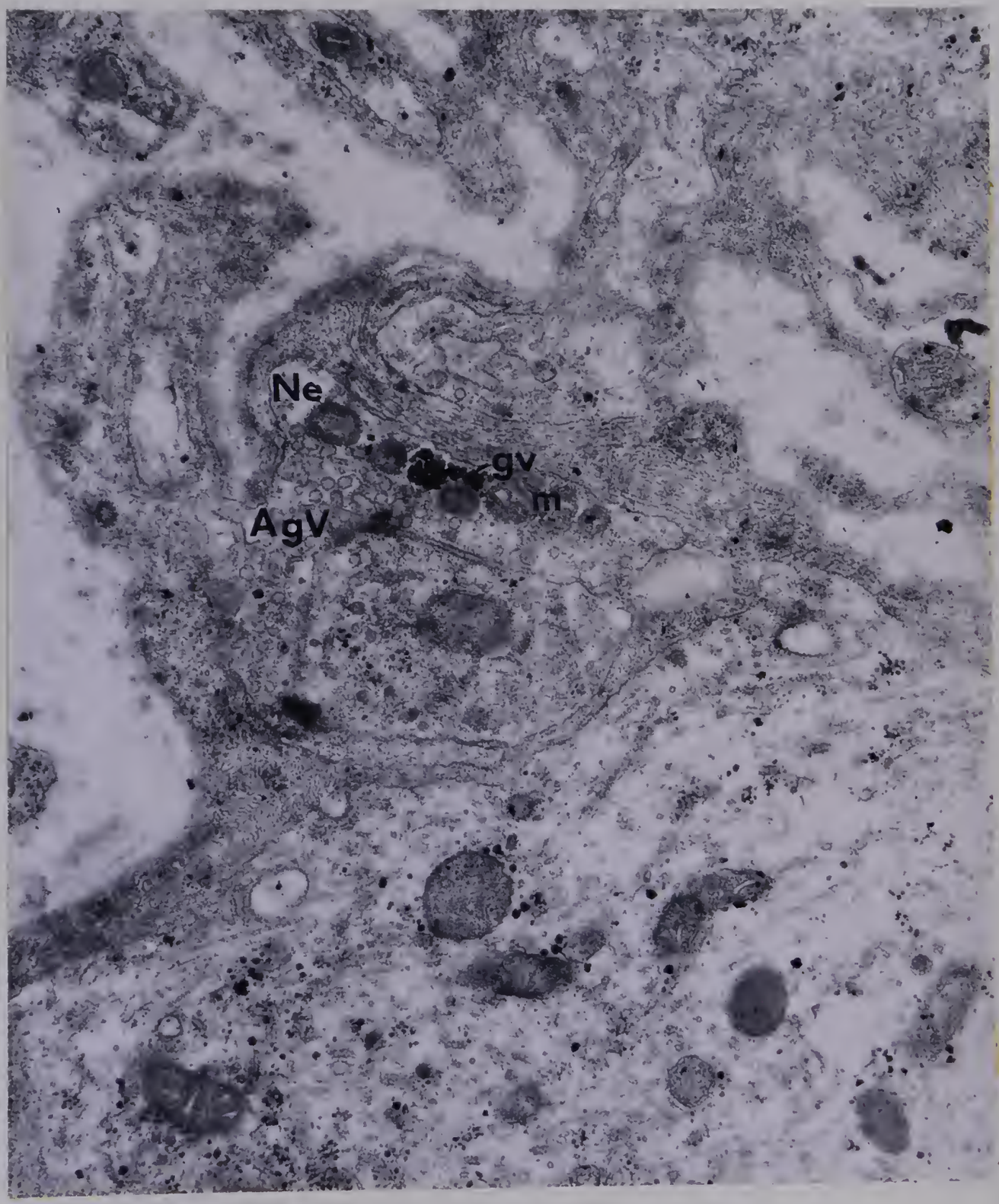
32

Figure 33. An electronmicrograph of a nerve ending containing a group of agranular vesicles, mitochondria and a closely packed group of granular vesicles.

Nerve ending	Ne
Agranular vesicles	AgV
Mitochondria	m
Granular vesicles	gv

Lead citrate and uranyl acetate stain.

Magnification x 34,500.



33

Figure 34. A low magnification electronmicrograph of a dendrite leaving a ganglion cell. Around it are seen a number of vesicle-packed nerve processes, some with only granular vesicles, some with agranular vesicles.

Dendrite	A
Ganglion cell	GC
Nerve processes	Np
Granular vesicles	gv
Agranular vesicles	AgV

Lead citrate and uranyl acetate stain.

Magnification x 11,000.

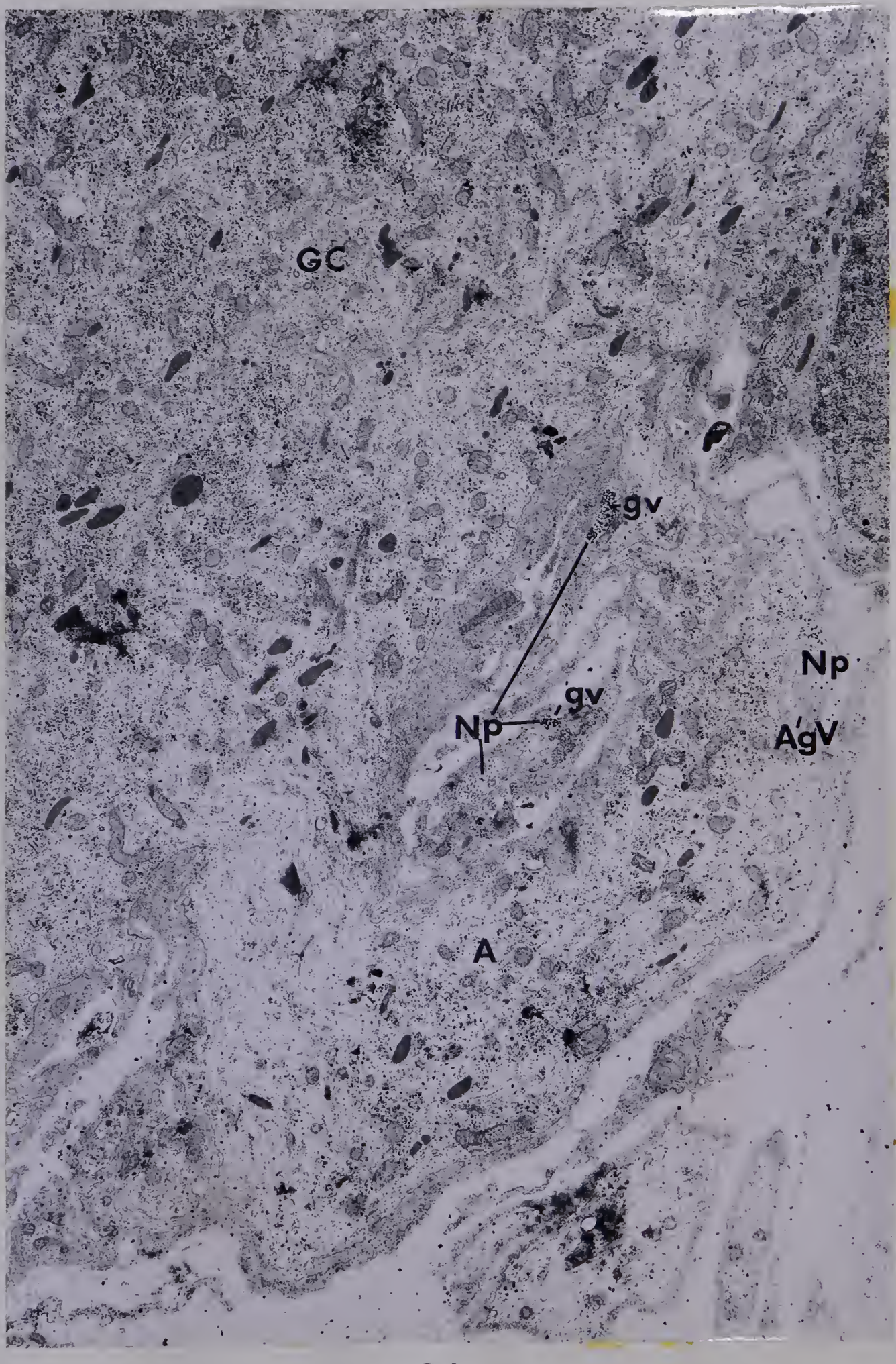

GC
gv
Np
gv
Np
AgV
A

Figure 35. An electronmicrograph of nerve processes with granular vesicles and agranular vesicles.

Nerve ending	Ne
Granular vesicles	gv
Agranular vesicles	AgV

Lead citrate and uranyl acetate stain.

Magnification x 34,500.

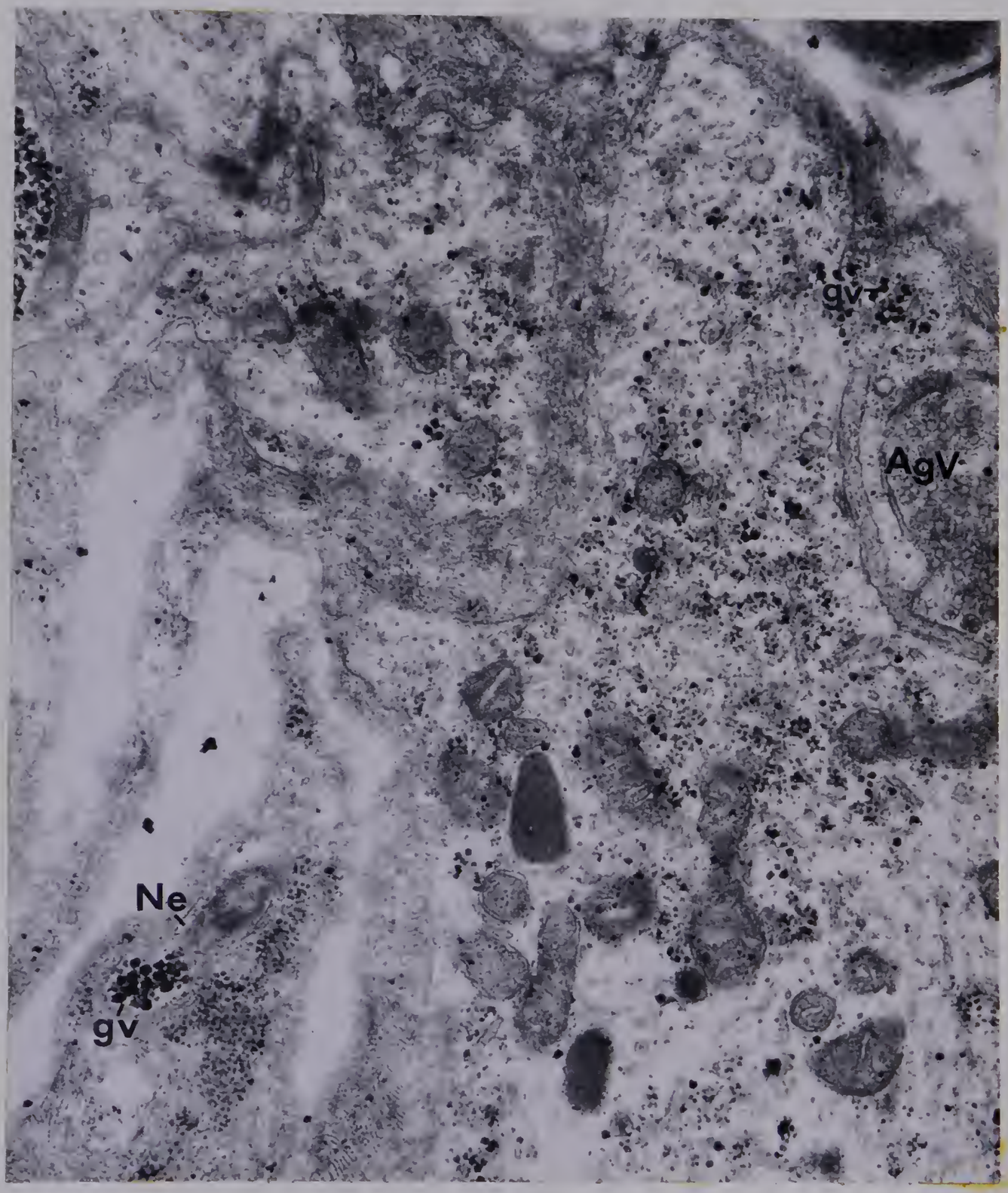
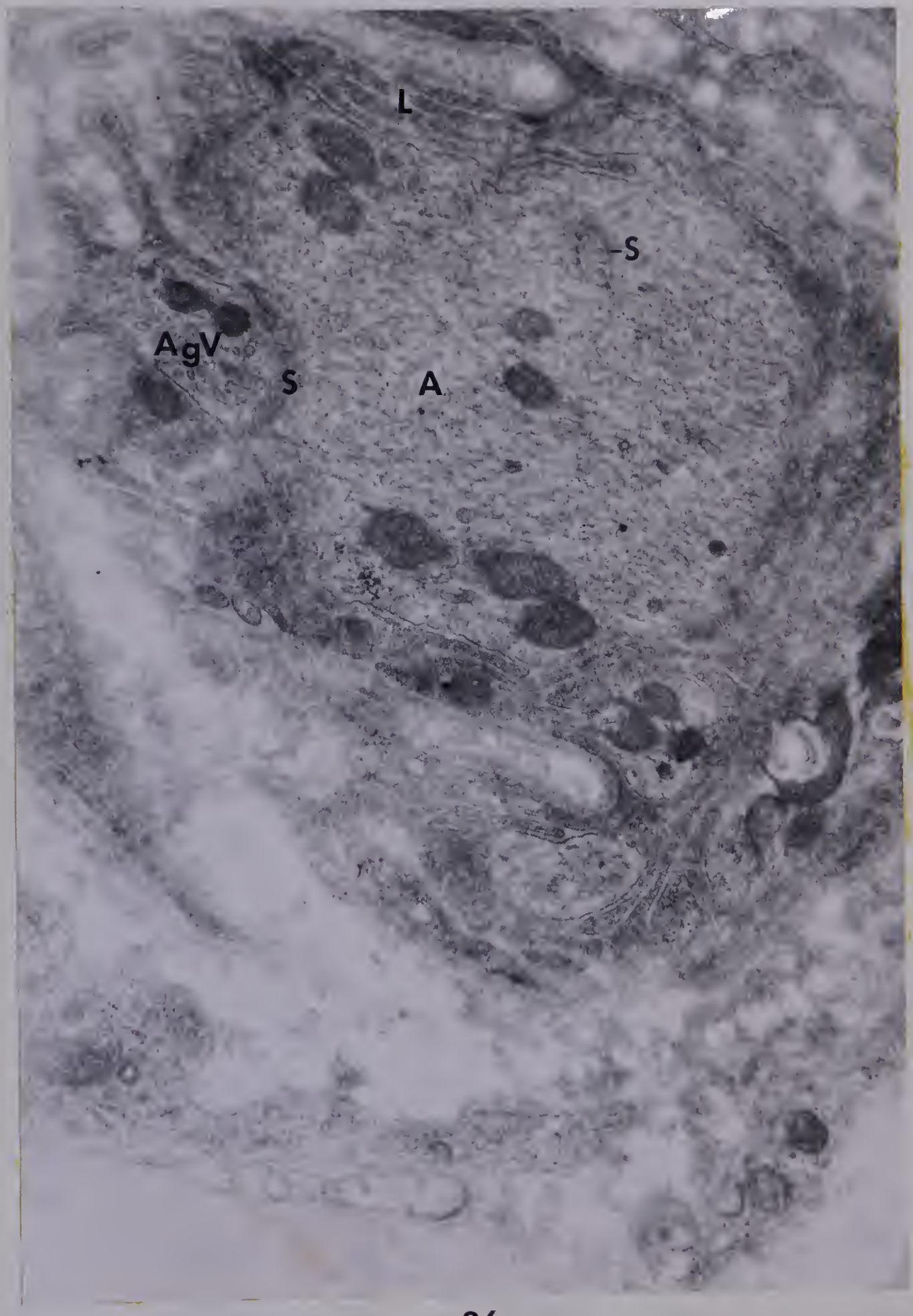

gv
AgV
Ne
gv

Figure 36. An electronmicrograph of an axon terminal containing agranular vesicles in synaptic contact with a process. Layers of Schwann cell cytoplasm can be seen around the nerve processes and fibers. A second synapse can be seen in the cytoplasm.

Agranular vesicles	AgV
Synapse	S
Axon	A
Layers	L

Lead citrate and uranyl acetate stain.

Magnification x 42,400.


L
S
AgV
S
A

B29991